EFFECTS OF AUTOXIDATION ON ANTIACRODYNIC POTENCY OF FATS AND LINOLEIC ESTERS [1]

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TWO FIGURES

(Received for publication May 29, 1948)

Studies on the nutritive value of rancid fats have been carried out under two types of dietary regimes. One, by Burr and Barnes ('43), involved the ability of a rancidified high fat diet to sustain growth. The other, by Quackenbush ('45), involved the ability of small amounts of rancidified fatty acids to cure acrodynia. In the latter case, peroxide values were used as an indicator of the state of oxidation. However, Farmer ('46) has pointed out that peroxides disappear as oxidation and polymerization proceed. High peroxide values therefore do not necessarily reflect the most advanced stages of oxidation.

In a recent review Quackenbush ('45) stated that the literature contained a number of reports of malnutrition resulting from rancid dietary fat. In some instances the malnutrition has been traced to the destruction of dietary essentials such as the fat-soluble vitamins (Quackenbush, Cox and Steenbock, '42) and water-soluble vitamins (Pavcek and Shull, '42).

[1] This research was undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the War Department.

[2] Contribution No. 363 from the Department of Chemistry, Kansas State College, Manhattan.

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However, Quackenbush ('45) has stated that until experimental evidence provides the full explanation it must be assumed that rancid fats are able to exert a direct toxic effect. As increasing quantities of beef, pork, and poultry are being kept in cold storage, it might be desirable to know whether the rancid fats which are formed during this storage period (Schrieber et al., '47; Wagoner et al., '47) lose nutritional value.

In the present study, the ability of small quantities of oxidized fats and fatty acids to cure acrodynia was used as a means of testing their nutritive value. As pyridoxine and pantothenic acid (Birch, '38; Quackenbush, Steenbock, Kummerow and Platz, '42), and tocopherol (Hove and Harris, '46) have been found to be functional in fat deficiency, these dietary factors were fed to some of the rats in the various groups. Furthermore, the effects of these factors and the effects of oxidized fats on the characteristics of the mixed fatty acids deposited in the tissues were determined by means of spectrophotometric analysis.

MATERIALS AND METHODS

Preparation of materials

The ethyl and methyl linoleate were prepared from corn oil [3] via the tetrabromide according to the method of Rollet ('09), distilled under high vacuum and kept under an atmosphere of nitrogen at −13°C. The various esters listed in table 1 were prepared as follows: The rancidified methyl linoleate of high peroxide value was prepared by bubbling tank oxygen through 10 gm of the freshly distilled ester at 80°C. for three hours. The drastically oxidized ethyl ester was prepared by bubbling tank oxygen through the free acid for 20 instead of 3 hours. This acid was then esterified in absolute ethyl alcohol. This resulting material was extracted with Skellysolve F, washed with water, dried over sodium sulphate and

[3] Furnished through the courtesy of Dr. R. A. Baldwin, Corn Products Refining Co., Argo, Illinois.

freed from solvent; on standing, the product gradually became viscous in character.

The alkali-conjugated ethyl linoleate was prepared from freshly-distilled ethyl linoleate. The ester was isomerized with potassium hydroxide in ethylene glycol at 180°C. for 30 minutes (Mitchell, Kraybill and Zscheile, '43), poured over chipped ice and acidified with concentrated hydrochloric acid. The resulting mixture of conjugate isomers was then converted to the ethyl esters. The 10, 12 ethyl linoleate was prepared from dehydrated castor oil [4] by the method of Von Mikusch ('42). The 10, 12 acid was isolated from the mixed

TABLE 1

Characteristics of supplements

SUPPLEMENT	AMOUNT FED DAILY	IODINE VALUE	PEROXIDE VALUE	SPECIFIC ABSORPTION COEFFICIENT AT 2340 Å
	mg			
Fresh ethyl linoleate	25	167	9	0.3
Rancid methyl linoleate	25	109	629	0.6
Drast. oxid. ethyl linoleate	25	49	76	2.3
Conjugated ethyl linoleate	25	145	5	84.6
10, 12 ethyl linoleate	25	115	37	105.0
Rancid turkey fat	110	79	63	1.4
Fresh turkey fat	110	78	5	0.3

fatty acids by repeated fractional crystallization from Skellysolve F, ethyl alcohol and diethyl ether. The resulting product, which had a melting point of 55–56°C., was then converted to the ethyl ester. The rancid turkey fat was extracted from the skin of turkeys which had been kept in cold storage for two years; the fresh fat was extracted from the skin of freshly-killed birds. The skin was extracted with Skellysolve F, and the extract washed with water, dried over sodium sulphate and freed from solvent. The birds had been broad-breasted bronze turkeys of the same strain and had been kept on a standard turkey ration (Kummerow et al.,

[4] Furnished through the courtesy of O. Eisenschiml, Scientific Oil Compounding Co., Chicago.

'48). In each case the skin fats contained approximately the same percentage of mixed fatty acids, i. e. 20% linoleic, 50% oleic, 30% saturated, 0.1–0.5% arachidonic and no linolenic acid.

The various preparations were kept at —13°C. under nitrogen except during use. They were fed to the rats by means of a calibrated medicine dropper.

Preparation of animals

The diet and preparation of the animals were similar to those described by Quackenbush et al. ('39).

The diet was prepared as follows: 200 mg thiamine hydrochloride and 400 mg of riboflavin were dissolved in N/50 acetic acid, mixed with 18 pounds of casein [5], dried and then mixed with 78 pounds of glucose (cerelose) and 4 pounds of Wesson salts in a standard feed mixer. The animals [6] were supplemented once a week with one drop of fish liver oil [7] containing 250 I.U. of vitamin A [8] and 100 I.U. of vitamin D [9] per drop.

On this diet animals with an initial weight of 40–50 gm developed an acute acrodynia in 7–8 weeks. The severity of the acrodynia was recorded as a dermal index (Quackenbush et al., '39). This index was a numerical expression of the stage of development of lesions in the lips, eyelids, forepaws, hindpaws, ears and tail, and increased with the severity of the acrodynia.

Thirteen groups of three rats each were fed the supplements listed in table 2 for a three-week period. The rats were then killed, and each group saponified with 30% aqueous

[5] Vitamin test casein, purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

[6] Three weeks of age, obtained from Sprague-Dawley and Co., Madison, Wisconsin.

[7] Residue oil obtained on molecular distillation.

[8] Concentrate, obtained through the courtesy of Dr. Hickman, Distillation Products, Inc., Rochester.

[9] Crystalline, obtained from Dr. J. Waddell, E. I. du Pont de Nemours and Co., New Brunswick.

TABLE 2

Effects of fat supplements on dermal lesions and carcass fat

GROUP	FAT SUPPLEMENTS	MEAN BODY WEIGHT CHANGE IN 3 WEEKS	DERMAL INDEX		EFFECT ON DERMAL LESIONS	MIXED FATTY ACIDS		IODINE VALUE
			Initial	3 wks.		Mean weight		
		gm				*gm*	%	
I. Without pyridoxine and calcium pantothenate								
1	None	— 6.5	5.0	6.0	Negative	0.77	1.3	86.8
2	Conjug. et. linoleate	+ 1.7	5.7	3.0	Improvement	1.40	2.0	77.9
3	Drast. oxid. et. linoleate	— 9.0	4.0	7.0	Negative	0.85	1.4	93.5
4	10, 12 ethyl linoleate	— 11.3	4.0	7.3	Negative	1.58	2.0	85.6
5	Fresh turkey fat	+ 6.7	5.0	2.0	Curative	1.47	2.2	82.2
6	Rancid turkey fat	+ 4.0	4.3	2.7	Improvement	1.87	2.5	83.9
7	Fresh me. linoleate	+ 7.0	4.4	2.0	Curative	1.46	2.3	86.4
8	Rancid me. linoleate	0.0	4.5	2.5	Improvement	1.50	2.1	77.8
9	Rancid me. linoleate + tocopherol [1]	— 1.0	4.0	3.0	Improvement	2.14	3.0	80.3
10	Rancid me. linoleate + ethanolamine [2]	— 9.0	5.0	5.7	Negative	1.46	1.9	84.7
II. With 20 μg pyridoxine and 50 μg cal. pantothenate daily								
11	None	+ 43.0	4.3	4.3	Improvement	3.54	3.0	70.6
12	Rancid turkey fat	+ 68.0	4.3	1.3	Curative	12.23	9.0	64.8
13	Rancid methyl linoleate	+ 35.3	4.7	1.7	Curative	5.09	4.5	69.9
14	10, 12 ethyl linoleate	+ 33.5	4.7	3.4	Improvement	7.02	7.2	65.6

[1] Tocopherol — 14.0 mg per week.

[2] Ethanolamine — 18.0 mg daily.

alkali as described by Quackenbush and Steenbock ('42). The hydrolysate was freed of non-saponifiable material by extraction with Skelly-solve F and acidified with diluted hydrochloric acid (1:1). The crude fatty acids were extracted with Skelly-solve F, washed thoroughly with water, dried over sodium sulphate, freed from solvent and weighed. Io-

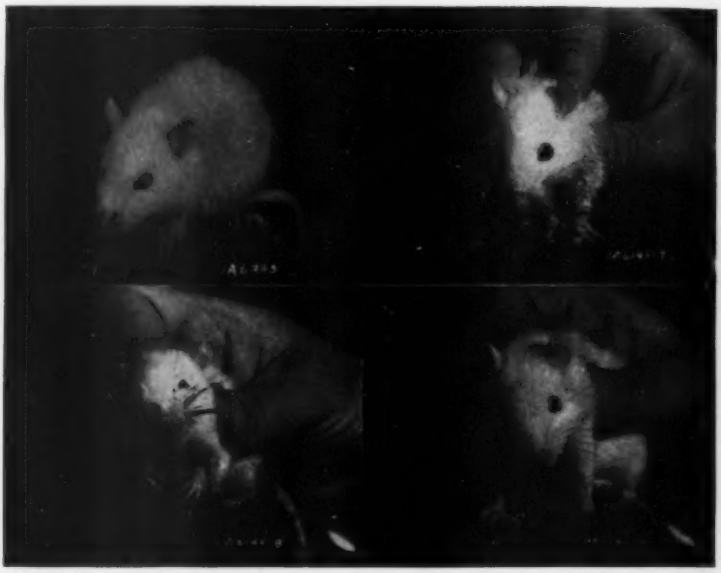

Fig. 1 Control 1A6293; rancid methyl linoleate 1A6195–7; 10, 12 ethyl linoleate 1A6195–8 and rancid methyl linoleate plus pyridoxine and calcium pantothenate 1A6195–5.

dine values were determined according to the method of Yasuda ('31) and the composition of the mixed fatty acids by the spectrophotometric method of Brice et al. ('45).

EXPERIMENTAL RESULTS

Effects on acrodynia

The results indicated that drastically oxidized or 10, 12 ethyl linoleate were not effective curative agents (table 2).

In fact, a more acute acrodynia and a greater decrease in body weight were noted in the animals of these groups than in those which had received no supplement (fig. 1). The alkali-conjugated ethyl linoleate, the rancid methyl linoleate of high peroxide value and rancid turkey fat alleviated the symptoms but were not so effective as the freshly-distilled

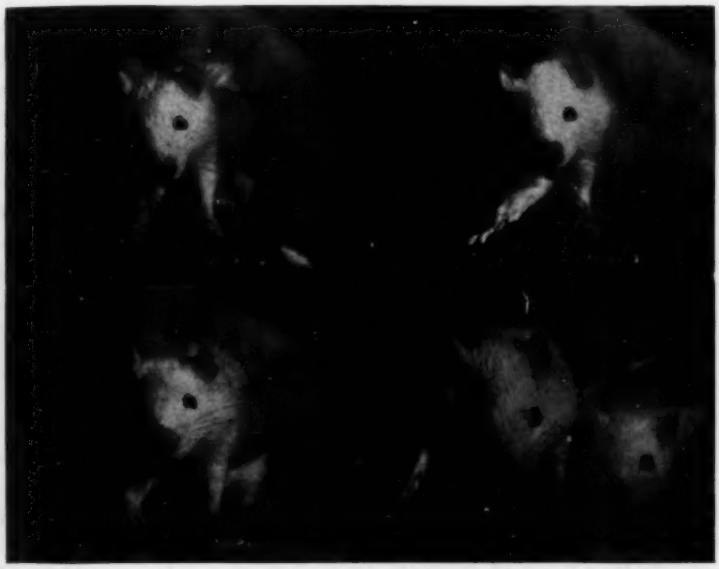

Fig. 2 Fresh turkey fat 1A6195–4; rancid turkey fat 1A6195–3; rancid turkey fat plus pyridoxine and calcium pantothenate 1A6195–1; rancid turkey fat (right) and rancid turkey fat plus pyridoxine and calcium pantothenate (left) 1A6195–12.

ester or fresh turkey fat (fig. 2). Topocopherol did not improve the effectiveness of rancid ethyl linoleate, and ethanolamine aggravated the symptoms.

When pryidoxine and calcium pantothenate were fed in addition to rancid methyl linoleate, the dermal symptoms were alleviated. However, the animals on these rations gained less weight than those whose diets had been supplemented with only pyridoxine and calcium pantothenate (table

2). This growth-depressing action of rancid methyl linoleate was also noted by Gyorgy et al. ('42). Pyridoxine and calcium pantothenate added to the curative value of rancid turkey fat but had little effect on the curative value of 10, 12 ethyl linoleate.

Effects on characteristics of extracted fatty acids

The presence of pyridoxine and calcium pantothenate, rather than the presence of rancid or fresh fat, seemed to have the most influence on the quantity and character of the fat deposited (table 2). Animals fed pyridoxine and calcium panothenate in addition to rancid fat deposited from two to three times more fat than those which had received only rancid fat, or 4.5–9.0 and 2.0–2.5% respectively. Except for the animals which had been fed drastically oxidized ethyl linoleate, the percentage of fat extracted from the animals fed fresh or rancid fat varied only 0.5%, or from 2.0–2.5%. The drastically oxidized linoleate was probably not absorbed readily from the intestinal tract, as these animals contained only 0.2% more fat than those which had received no supplement.

The iodine values of the fats decreased from 8 to 20 points when pyridoxine and calcium pantothenate were fed in addition to fat (table 2). The fat extracted from the animals which had received no fat supplements or drastically oxidized ethyl linoleate had the highest iodine value, or 86.8 and 93.5 respectively. Those which had received pyridoxine and calcium pantothenate in addition to rancid turkey fat or 10, 12 ethyl linoleate had the lowest iodine value, or 64.8 and 65.6, respectively.

Spectrophotometric analyses of the mixed fatty acids indicated that the percentages of essential fatty acids in the tissues were not adversely affected by the feeding of rancid fat (table 3). The animals which had been fed only rancid turkey fat contained the highest, and those which had received no supplement contained the lowest, percentages of linoleic acid, or 7.2 and 4.0%, respectively.

TABLE 3

Spectrophotometric studies of mixed fatty acids from carcasses of rats fed various fats

GROUP	SUPPLEMENT	SATURATED		OLEIC		LINOLEIC		ARACHIDONIC	
		%	mg [1]	%	mg [1]	%	mg [1]	%	mg [1]
I. Without pyridoxine and calcium pantothenate									
1	None	22.9	295	67.5	870	4.0	52	5.6	72
2	Conj. et. linoleate	33.8	675	54.8	1096	6.3	126	5.1	102
3	Drast. oxid. et. linoleate	20.5	287	67.3	942	5.1	71	7.1	99
4	10, 12 et. linoleate	24.0	480	65.1	1301	6.2	124	4.7	94
5	Fresh turkey fat	27.5	605	61.8	1360	6.1	134	4.6	101
6	Rancid turkey fat	24.9	623	63.9	1597	7.2	180	4.0	100
7	Fresh me. linoleate	25.5	586	62.5	1433	6.5	149	5.5	127
8	Rancid me. linoleate	33.7	707	55.8	1172	4.8	101	5.7	120
9	Rancid me. linoleate + tocopherol	26.0	780	65.3	1960	4.8	144	3.9	117
10	Rancid me. linoleate + ethanolamine	23.4	445	66.5	1263	5.8	110	4.3	82
II. With pyridoxine and calcium pantothenate									
11	None	27.8	833	68.5	2055	2.0	60	1.0	30
12	Rancid turkey fat	34.8	3130	60.9	5475	2.6	234	1.1	99
13	Rancid me. linoleate	28.5	1282	68.0	3060	2.0	90	1.5	67
14	10, 12 et. linoleate	21.2	1527	76.1	5480	1.4	101	0.9	64

[1] Mean per 100 gm of carcass. No linolenic acid was present except for traces in groups which received pyridoxine and calcium pantothenate.

Less than a 3.5% variation between groups was noted in the total linoleic and arachidonic acid content. The animals which had been supplemented with drastically oxidized ethyl linoleate contained the highest, and those supplemented with rancid ethyl linoleate contained the lowest, percentage of total essential fatty acids, or 12.2 and 8.7% respectively. When pyridoxine and calcium pantothenate were also fed, the total essential fatty acids varied from 2.3 to 3.7%. The total percentage was therefore only one-third as large as in the absence of pyridoxine and calcium pantothenate. However, comparisons based on percentages alone might be misleading, as the animals which had received pyridoxine and calcium pantothenate had greatly increased in weight. Therefore, comparisons based on the mean weight of fatty acid per 100 gm of tissue were made, in order to reflect differences between groups more accurately.

From these data it can be seen that in every case the animals which had received pyridoxine and calcium pantothenate synthesized substantial amounts of oleic and saturated fatty acids. Furthermore, the animals which had received rancid methyl or 10, 12 ethyl linoleate as well as pyridoxine and calcium pantothenate contained from 1,005 to 3,425 mg more oleic and 449 to 694 mg more saturated fatty acid than the control groups. The quantity of total essential fatty acids was not increased even though approximately 500 mg of linoleate was fed to these animals during the assay period. It is possible that pyridoxine or calcium pantothenate served as biological antioxidants or helped to convert rancid or conjugated acid to oleic and saturated fatty acid.

DISCUSSION

The nutritive value of rancid fats was improved when the diets of the experimental animals were supplemented with pyridoxine and calcium pantothenate. As yeast contains these two vitamins, its addition to the dietary regime (Burr and Barnes, '43) could influence the ability of a rancidified high fat diet to sustain growth. Whipple ('33), for example, has

shown that rats fed oxidized lard resumed growth when their diets were supplemented with additional quantities of ether-extracted yeast. It is interesting to note, however, that in the present study it was found that 10, 12 ethyl linoleate was not curative in the presence of pyridoxine and calcium pantothenate. Burr ('42) has shown that 9, 11 linoleic acid also lacks curative properties under these conditions.

Farmer ('46) and others have shown that in the first stages of autoxidation of linoleic acid the double bonds are conjugated with the simultaneous formation of hydroperoxides. During the later stages of autoxidation over 70% of the double bonds have been reported to be conjugated (Bolland and Koch, '45). In agreement with Burr ('42), the present study indicates that fats in the first stages of autoxidation alleviated the symptoms, but were not so effective as fresh fats. Fats in the later stages of autoxidation as represented by 10, 12 and drastically oxidized ethyl linoleate respectively were non-curative.

The various supplements of linoleic acids seemed to have been metabolized or converted to oleic and saturated fatty acids, although less fresh than rancid or 10, 12 linoleate was converted. When pyridoxine and calcium pantothenate were fed in addition to fat supplements, the oleic and saturated fatty acid contents of the tissues were greatly increased. However, these fatty acids must have been synthesized from carbohydrate or protein, as increased amounts of them were also found in the animals which had received no fat supplement.

It is possible that the availability of pyridoxine and pantothenic acid is a limiting factor in rat acrodynia. Umbreit and Gunsalus ('45) have shown that pyridoxine functions in a coenzyme system in amino acid metabolism. When pyridoxine is present in suboptimal amounts, it may not be able to function in both the metabolism of unsaturated fatty acids and the building of protein. The present study indicates that in extreme cases undesirable fats seem to have a priority claim,

so to speak, on the pyridoxine already stored in the tissues, as the animal actually lost weight and the symptoms were aggravated. Whether pantothenic acid is also necessary was not brought out in this investigation. However, Quackenbush, Steenbock, Kummerow and Platz ('42) indicated that both pyridoxine and pantothenic acid are functional in rat acrodynia.

The high peroxide value and the small amount of conjugated fat in the rancid turkey fat indicate that only the first stages of oxidation are involved during the cold storage of poultry. The rejection by a taste panel of cooked meat which contained rancid fat (Kummerow et al., '48) may have had a physiological basis. However, as the stored meat probably contained substantial amounts of pyridoxine and pantothenic acid, the present data indicate that ingestion should have caused no harmful effects.

SUMMARY

The ability of small quantities of oxidized fats to cure acrodynia was used as a means of testing their nutritive value. The results indicated that rancid methyl linoleate or the fat extracted from turkeys which had been subjected to cold storage for two years alleviated the symptoms but were not as effective as the freshly distilled ester or fresh turkey fat. Tocopherol did not improve the effectiveness of rancid ethyl linoleate and ethanolamine aggravated the symptoms. Drastically oxidized fats or 10, 12 ethyl linoleate were not effective curative agents.

When pyridoxine and calcium pantothenate were fed in addition to rancid fat the dermal symptoms were alleviated. This response was not due to pyridoxine or calcium pantothenate alone, but to a combination of the fat- and water-soluble factors. Spectrophotometric analysis of the carcass fats indicated that the various supplements of oxidized linoleic esters were converted to oleic and saturated fatty acids. Pyridoxine and calcium pantothenate seemed to be functional in this process.

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THE NUTRITIVE VALUE OF SOME LEGUMES. LATHYRISM IN THE RAT. THE SWEET PEA (LATHYRUS ODORATUS), LATHYRUS SATIVUS, LATHYRUS CICERA AND SOME OTHER SPECIES OF LATHYRUS [1]

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ONE FIGURE

(Received for publication May 14, 1948)

The toxicity of certain species of *Lathryus* and related legumes for man and many domestic animals has been recognized since early in the eighteenth century (Schuchardt, 1886–87). Outbreaks of lathryism in man in India have been studied extensively by Stockman ('29, '31, '34a, '34b), and Shourie ('45), and in Spain by Díaz and his collaborators ('41, '42, '43), who have investigated lathyrism in that country from both the clinical and experimental viewpoints. Important in the production of lathyrism in man are *Lathyrus sativus, clymenum,* and *cicera.* The peas, which are usually eaten by the poorer classes in India and Spain only when the supply of cereal grains is deficient, must constitute a significant part of the diet, one-third to one-half, according to Stockman, ('29), in order to cause the disease. Males are more susceptible than females, an incidence of 20 males to one female be-

[1] Grants in aid from the Nutrition Foundation of New York and the Faculty Research Fund of the Horace H. Rackham School of Graduate Studies of the University of Michigan have made possible this and related studies of the nutritive value of peas of the genus *Lathyrus.*

ing reported in one community by Díaz ('41). The symptoms (notably muscular weaknes and paralysis of the extremities) have suggested to many the presence of a neurotoxin which may cause a degeneration of the spinal cord. The pathological study of a case of human lathyrism *(L. sativus)* has shown severe injury to the nervous system (Filimonoff, '26).

Marked variations in the susceptibility of domestic animals to *Lathryus* pea intoxication have been observed (Stockman, '29). It is usually stated that lathyrism *(Lathyrus sativus* and *cicera)* cannot be produced experimentally in the white rat (Visco, '23, '24a, '24b; McCarrison, '28; Zagami, '31; McCarrison and Krishman, '34; Díaz and Vivanco, '42). Geiger, Steenbock, and Parsons ('33), however, observed lathyrism in the rat after the flowering sweet pea *(Lathyrus odoratus)* was fed in considerable amounts. This species of *Lathyrus* has also been associated with human lathyrism in Spain (Díaz, '41). The data on the nutritive properties of *Lathryus* species are somewhat contradictory; this may be due in part to local names for the various species and the resultant confusion in their identification, as pointed out by Schuchardt (1886–87), Díaz and Vivanco ('42), and others.

We have studied in the rat, the nutritive values and toxicities of a number of species of *Lathryus,* including two which are commonly associated with the condition of lathryism in man, *L. sativus* and *L. cicera,* and also including *L. odoratus.* The results of this last study, which confirm and extend the observations of the Wisconsin group (Geiger, Steenbock, and Parsons, '33), have been published in preliminary form. (Lewis and Esterer, '43).

In addition, with the cooperation of Mr. Roland McKee of the Bureau of Plant Industry of the United States Department of Agriculture and his associates, we have been able to study five other species which have been of some interest in various parts of the United States, chiefly because of their usefulness as forage crops. Many of the *Lathyrus* species can be grown under conditions which are difficult or unsatisfactory for the production of other forage crops. We wish to

express our sincere appreciation to Mr. McKee and his associates for this help, which has made it possible to extend this study beyond the scope which was originally projected.

EXPERIMENTAL

Young white rats, 50 to 80 gm in weight, in litter units, were paired as to sex and weight. One animal of each pair received the experimental *(Lathyrus)* diet ad libitum, while the food consumption of the paired litter mate fed the control (edible pea) diet was limited by the food intake of the mate fed the experimental diet. Daily records of food consumption were kept.

Finely ground meal (20 mesh sieve) was prepared from the *Lathyrus* peas and, for the control diets, from the edible split white peas of commerce *(Pisum sativum, var. arvense)*. Since it is known that many legumes supply protein of inferior biological value, 10% of casein, a protein of high biological value, was incorporated into all the diets. The pea meal was added at a level of 50% to insure a high content of the potentially toxic legume. The protein content ($N \times 6.25$) of the diets fed varied from 21.55% (edible white pea) to 26.9% *(Lathyrus cicera)*, of which 10% was derived from the casein. With *Lathyrus tingitanus,* which because of its toxicity was fed at the 25% level, the total dietary protein was 19.57%. It is not believed that the differences in protein content of the diets representing various *Lathyrus* species (22.55–26.95%) were significant, particulary since, as will be noted later, best growth was obtained with those containing *L. sativus,* which were lowest in protein content of the rations containing *Lathyrus.*

The mixed diets were constituted as follows: pea meal, 50%; casein[3], 10%; corn starch, 27%; sucrose, 5%; salt mixture (modified Osborne–Mendel), 4% corn oil, 2%; and cod liver oil, 2%. The dry ingredients of the diet were thoroughly mixed and stored, and the oils added to the mixture, to make

[3] Labco, vitamin-free.

the complete diets as needed. In addition, each rat received 200 mg of dried yeast and, in later experiments, 100 mg of powdered liver extract [3], which were added to the diet to supply the B factors in addition to the vitamins made available in the dried yeast.

Sources of legumes

1. The edible split white peas used for the control diets were supplied by Mr. Floyd Trail of the Washburn-Wilson Seed Company, Moscow, Idaho.

2. *Lathyrus odoratus.* The sweet pea seed was made available through the generosity of Mr. Raymond H. Coulter of the Ferry-Morse Seed Company, Detroit.

3. *Lathyrus sativus.* The seed was obtained from India through the cooperation of Dr. B. Mukerji of the Biochemical Standardization Laboratory, Calcutta. The seed was submitted to Mr. Paul Russell and Mr. Roland McKee of the Bureau of Plant Industry who, after examination of the seed and of young plants grown in greenhouse experiments, reported that the material was one of the "small seeded dark strains of *L. sativus;*" the close resemblance of this seed to that of certain strains of *L. cicera* was, however, noted.

4. *Lathyrus cicera.* Two samples of seed, grown in plantings of 1942 and 1943 at College Station, Texas, were obtained from Mr. Karl F. Manke and Mr. P. R. Johnson.

5. *Lathyrus hirsutus.* Two samples of the seed of this species, grown during different seasons, were obtained from Mr. Roland McKee, who stated that "this is the only species that is being grown extensively in the United States. This species is quite widely spread in the states of Louisiana, Mississippi, Alabama and adjacent territories" and "has been harvested and sold as a pasture plant."

6. *Lathyrus tingitanus.* Two samples of seed, grown in different seasons by the Division of Forage Crops and Diseases at Corvallis, Oregon, were obtained through Mr. H. A. Schoth.

[3] Lilly.

7 and 8. *Lathyrus aphaca* and *Lathyrus sphaericus*. Seeds of these species were secured through Mr. McKee.

9. *Lathyrus sylvestris Wagneri*. Three samples of seed, two grown in different seasons at the Western Agricultural Experiment Station at Puyallup, Washington, and one grown at Corvallis, Oregon, were obtained through Mr. J. W. Daniels, Mr. D. Dickson, and Mr. Schoth, respectively. A sample of the cured foliage ("hay") was also received from Mr. Schoth. This pea ("flat pea") has been recommended as a promising pasture crop on rough or logged-off land (Grunder and Dickson, '48).

DISCUSSION

Lathyrus odoratus

As shown in table 1, rats fed a diet which contained 50% of sweet pea meal uniformly developed lathyrism. The symptoms were, for the most part, similar to those reported by Geiger, Steenbock, and Parsons ('33) and included incontinence, lameness, paralysis of the rear limbs, paralysis of the front limbs with loss of control of the wrists, and spinal curvature. The spinal curvature was observed chiefly in the thoracic region and was usually both ventral and lateral. The sternum was deformed, frequently to such an extent that the volume of the thoracic cavity was strikingly diminished. We observed no instances of the hernias[4] reported by Geiger, Steenbock, and Parsons ('33). It was striking that, despite the markedly toxic character of the diet, the animals in most cases continued to consume the diet readily, almost to the point of death. There was a marked variation in the period which elapsed from the onset of symptoms (initial, 2 to 7½ weeks; marked paralysis, 3 to 14 weeks) to death. All of our animals fed the sweet pea diet succumbed, but the period of feeding varied from 5 to 35 weeks. Most animals died within 7 to 10

[4] In very recent experiments still in progress, in a series of 6 animals, we have observed one hernia in which a testis was involved. No hernias were observed in any of the animals referred to in table 1, nor in the subsequent experiments in which other species of *Lathyrus* were fed.

weeks. None of the control rats showed any symptoms or abnormalities.

We used young rats in our experiments, as already indicated. However, we were able to produce the same clinical picture with older rats (up to 250 gm body weight). The spinal curvatures were, however, much less marked and the time when the symptoms appeared was much less constant than with the young rats.

TABLE 1[1]

Increments in weight and clinical observations (paralysis) of young white rats fed sweet pea, modified sweet pea and edible pea (control) diets

GROUP	DIET	AVERAGE GAIN PER 100 GM FOOD	CLINICAL
		gm	
1.	Odoratus (21)	20.6	7,8,5,5,8,10,11,10,5,7, 5,4,5,5,6,7,3,4,4,13,14
2.	White pea (16)	24.7	no paralysis
3.	Odoratus + 25% casein (2)	16.6	4,6
4.	White pea + 25% casein (2)	21.4	no paralysis
5.	Odoratus, baked (5)	14.7	7,7,6,7,8
6.	Odoratus, baked + 12 mg ascorbic acid daily (5)	15.7	7,5,5,6,6
7.	Odoratus, autoclaved (4)	26.4	4,5,5,6
8.	White pea, autoclaved (4)	31.4	no paralysis
9.	Water-extracted odoratus + split pea extract[2] (4)	20.2	no paralysis
10.	Water-extracted white pea + odoratus extract[2] (4)	25.8	4,5,7,5
11.	40% white pea + 10% dried odoratus extract[3] (2)	22.7	11,11
12.	Alcohol (30%)— extracted odoratus (2)	17.8	no paralysis
13.	White pea + 1% phytin (5)	25.1	no paralysis
14.	White pea (5)	23.8	no paralysis
15.	Odoratus + liver extract (3)	27.4	9,7,7

[1] The gains per 100 gm of food are calculated for the first 4 weeks of the experimental period. The values in parentheses are the number of experimental animals in each group. The figures in the column headed "Clinical" represent the period in weeks at which symptoms of *marked* paralysis appeared. The diets all included 50% of pea meal and 10% of casein except where indicated (groups 3, 4 and 11).

[2] Aqueous extract.

[3] 30% alcohol extract.

Through the cooperation of Dr. Fred J. Hodges and Dr. Robert W. Bryn of the Department of Roentgenology, it was possible to make a series of roentgenological studies at weekly intervals on a group of 6 rats fed the *Lathyrus* pea diet and a similar control group fed the white pea diet. We were thus able to observe the progressive changes in the skeletal structures as lathyrism developed (fig. 1). No path-

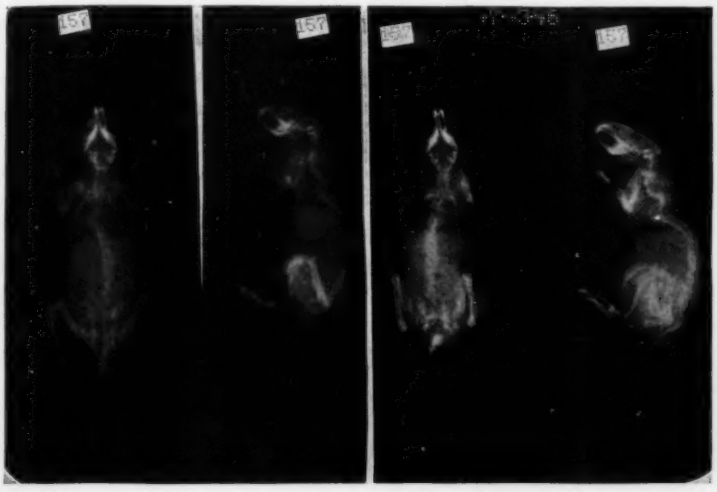

Fig. 1 Roentgenological observations on rat 157 fed the *Lathyrus odoratus* diet. The photographs at the left and right show the skeletal changes at 5 and 7 weeks respectively.

ological conditions were observed in the control group at any time.

The development of marked kyphosis and scoliosis of the spine with the apex of curvature in the mid- and lower-thoracic regions, observed in the skeletons at autopsy, was readily seen in the films. Curvature of the spine could be observed after two weeks on the sweet pea diet, and increased progressively during the period of observation. Although it would seem likely that there should be deformity in individ-

ual segments of the vertebrae due to compression, there appeared to be no gross evidence of such deformity or decalcification in the course of the development of the abnormal picture. The changes were postulated to be in the soft tissue-supporting structures of the spine, which were such that deformity could easily occur. The deformity of the bony thoracic cage was considered to be the result of the developed kyphosis of the spine.

Changes in the femur were similar to those seen in human osteomalacia. In the rats there occurred irregularities of the proximal third of the femur with thickening of the cortices, sub-periosteal calcification and multiple infractions near the end of the shaft. The changes in the tibia, for the most part, consisted of osteoperosis (demineralization). Most striking were the changes in the knee joints, with fragmentation of the epiphyses of the tibia, femur, and to a milder degree, of the patella. Marginal sclerosis was present and there was evidence suggestive of hemarthrosis (bleeding into the joint). The changes in the knee joints might be compared to those in Charcot's disease in man, which is a disorder of neurotrophic origin.

These *in vivo* observations were confirmed by autopsy. The changes in the spine, femur, and tibia were similar to those described by Geiger, Steenbock and Parsons ('33). The spinal column, bones of the hind limbs, and muscle were examined microscopically by Professor Carl V. Weller of the Department of Pathology, to whom we are indebted for the pathological reports. Characteristic findings on two animals are presented. In Rat 17, "the spine shows the same severe changes as are found in the long bones. There is a sharply localized collapse of the centra which must mean that there was an early decalcification. This has resulted in a compression of the spinal cord of so severe a degree that there is adequate explanation of the paralysis of the extremities. There is much new formation of connective tissue and cartilage and also periosteal hemorrhage. The proliferation of connective tissue extends into the neighboring muscle." In

Rat 5, the bone marrow, after decalcification, "is in part fatty myxomatous and there is much less hematopoiesis than in the control animal. There has been a new formation of bone upon the original cortex and a sclerotic layer has developed in the periosteum. Epiphyseal lines are widened, particularly at the upper end of the femur . . . . .In addition to the effect in hematopoiesis, there is a delayed ossification (such as is seen in rickets), while new formation of bone around the shaft is more suggestive of a late stage of scurvy."

The toxicity of the *Lathyrus* pea diets was not altered when extra casein was added to give a total dietary protein content of approximately 38% (25% casein), the extra casein replacing an equivalent amount of starch in the diet. The presence of considerable amounts of good animal protein did not delay or prevent the onset of lathyrism (cf. Groups 3 and 4, table 1). The failure of casein in concentrations of either 10 or 25% in the diet to prevent the development of lathyrism is of interest, since it has been suggested that lack of adequate amounts of dietary tryptophane may be a factor in lathyrism (Díaz, '41). Certainly the tryptophane content of our casein-pea meal diets was such that there could have been no tryptophane deficiency.

The sweet pea meal was mixed thoroughly with an equal weight of water and the mixture was baked in an oven at 80° for 24 hours. When the baked meal was incorporated into the diets, there was no change in the toxicity (Group 5, table 1). More drastic heat treatment was obtained by autoclaving both the sweet pea and white pea meals at 120° for 6 hours. Diets in which the autoclaved sweet pea meal was present (Group 7, table 1) were no less toxic than those which contained the raw meal. It is therefore evident that, as noted by Geiger, Steenbock and Parsons ('33), the toxic principle of sweet peas is thermostable, even when autoclaved. There is considerable evidence that cooking frequently increases the biological value of otherwise inferior leguminous protein (Waterman and Johns, '21; Johnson, Parsons, and

Steenbock, '39; Lewis and Taylor, '47). It is of interest to note the somewhat better gains per 100 gm of food consumed which were observed when the autoclaved pea meals, both the sweet and white pea, were fed (cf. Groups 1 and 7 and Groups 2 and 8, table 1).

In a further study of the properties of the toxic agent present in sweet peas, the meal was extracted overnight at 0° with distilled water. The extract was decanted and the extraction repeated three times. The combined extracts were evaporated to a thick syrup *in vacuo*. A similar extraction of white pea meal was carried out. To the extracted white pea meal residue was added the concentrated extract of sweet peas, and to the extracted sweet pea meal residue was added the extract of white peas. The combined extracts and residues were then dried in thin layers at 80°, finely ground, and the mixtures incorporated into the diets. All of the animals receiving the *Lathyrus* extract-white pea meal residue diet (Group 10, table 1) showed the characteristic symptoms of acute lathyrism, while none of the group fed the white pea extract-sweet pea meal residue diet (Group 9, table 1) exhibited any signs of toxicity. It was thus shown that the toxic material was readily extracted by cold water from the sweet pea meal and that the residual extracted meal was non-toxic, a finding in accord with that of Geiger, Steenbock and Parsons ('33) who used boiling water in their experiments. The toxic agent could not be removed by extraction of the meal with cold ether or 95% alcohol. After a series of trials, it was found that the most efficient removal of the toxicant could be effected by slow percolation of the meal with 30% alcohol in the large percolators commonly used for the extraction of drugs in pharmaceutical procedures. When sweet pea meal thus extracted was incorporated into the diet (Group 12, table 1), no toxicity resulted. On the other hand, when the dried 30% alcohol extract of sweet pea meal was added to white pea meal and the mixture incorporated into the diet (Group 11, table 1), the toxicity was similar to that observed

when a control group of animals was fed the unextracted sweet pea meal diet.

It has been suggested (Stockman, '34a, '34b) that an important factor in lathyrism and other intoxications caused by eating the seeds of certain legumes may be the presence of a toxic organic phosphate, possibly phytin or phytic acid, and that lathyrism results when this ester fails to be split by the phosphatase in the alimentary canal and is absorbed without hydrolysis. Although in the light of more recent studies of phytin this hypothesis seemed improbable, we tested it by feeding edible white pea diets to which 1% of phytin [5] had been added. Although the experiments were continued for a period longer than that required for the development of skeletal changes and other symptoms of lathyrism (14 weeks), no toxic effects were observed (Group 13, table 1), nor was the growth of the animals inferior to that of the control group fed the edible pea diet without added phytin (Group 14, table 1).

We have observed that the addition of liver extract to the diet of rats has frequently resulted in increased food consumption and improved growth. Powdered liver extract [6] in amounts of 100 mg per day was added to the diet of the rats fed the sweet pea meal. These animals were paired with rats fed the sweet pea diet alone, whose food consumption determined the amounts of food offered the group fed the liver extract. The gains per 100 gm of food for the initial 4-week period were 24.9, 14.8 and 19.2 gm for the *odoratus* group as compared with 26.0, 29.4 and 26.6 for the liver extract group. Despite this superior gain, the addition of powdered liver extract failed to protect against the onset of lathyrism (Group 15, table 1).

Both the roentgenological examination of the living animals and the pathological examination of the tissues indicated the presence of hemorrhagic changes, suggestive of scurvy.

[5] We wish to express our appreciation to the Staley Manufacturing Company for the gift of the phytin used in these experiments.

[6] See footnote 3, page 540.

Although the rat is able to synthesize ascorbic acid and does not require its presence in the diet, it was thought possible that the toxicant might in some manner have increased the ascorbic acid requirement, so that the animal was unable to synthesize it in adequate amounts. A condition such as this was observed in mice by Wolley and Krampitz ('43). This species also does not require dietary ascorbic acid, being able to meet its needs by synthesis. If glucoascorbic acid, a homologue of ascorbic acid containing 7 rather than 6 carbon atoms, were fed, the mice developed severe scurvy, comparable to the experimental scurvy seen in guinea pigs. These observations were interpreted as indicating a biological competition between the glucoascorbic acid and the ascorbic acid synthesized by the animal. The addition of liberal amounts of ascorbic acid to the *Lathyrus* diet afforded no protection against the production of lathyrism. Rats fed the sweet pea diet with or without added ascorbic acid (12 mg daily) developed lathyrism of essentially the same severity and within the same period of time (Groups 5 and 6, table 1). The content of ascorbic acid in the plasma and tissues [7] of a group of control animals which were fed the stock diet, and of the groups which received the sweet pea diet with and without added ascorbic acid, was determined. No differences in the values were observed (table 2). The ascorbic acid was determined by the Bessey ('38) modification of the procedure of Mindlin and Butler. It would appear that a disturbance in ascorbic acid metabolism is not a factor in the causation of lathyrism by the sweet pea diet used in the present study.

Two theories of the cause of lathyrism are commonly proposed: lathyrism may be a "deficiency" disease or it may be occasioned by the presence of an intoxicant (presumably a "neurotoxin") in the seeds of the *Lathyrus* species. Deficiencies in protein and in vitamins have been suggested. The poor biological value of the proteins of many legumes is recognized and, in particular, claims have been made that *Lathy-*

[7] These determinations were carried out by Mrs. Naomi Levine Foa, to whom we are indebted.

rus pea protein is deficient in tryptophane. In the present experiments, in which casein at a level of 10% (or, in a few animals, 25%) was present in the diet, a deficiency in or a low biological value of the protein, and certainly a low content of dietary tryptophane, can hardly be the basis of the nutritive failure of the animals. Moreover, heat treatment of the sweet pea meal, a treatment which frequently increases the biological value of leguminous proteins, failed to influence the development of lathyrism.

TABLE 2[1]

Ascorbic acid content of tissues of control rats and of rats fed the sweet pea diet with or without the addition of ascorbic acid

TISSUE	DIET		
	Stock	*Lathyrus pea*	
		No supplement	*Ascorbic acid*
Liver	0.24 (0.15–0.32)	0.19 (0.15–0.27)	0.21 (0.16–0.25)
Kidneys	0.10 (0.06–0.13)	0.12 (0.09–0.14)	0.13 (0.06–0.16)
Adrenals	2.36 (0.91–4.2)	2.86 (2.2–3.4)	2.61 (1.9–3.1)
Plasma	0.47 (0.22–0.96)	0.26 (0.14–0.38)	0.49 (0.35–0.61)

[1] Average values for three groups of 5 animals each are given with the ranges in parenthesis. All values are expressed as milligrams per gram of tissue or per 100 ml of plasma. The rats fed ascorbic acid received 12 mg per day.

Among vitamins, lack of which has been believed to be a factor in lathyrism, are vitamin A and the B complex group. In all our experiments, the rats received vitamins A and D in the form of cod liver oil. None of our control animals exhibited the symptons of lathyrism as did the animals fed the sweet pea diet, as might be expected if deficient fat-soluble vitamins were related to lathyrism. Water-soluble vitamins were supplied by dried yeast powder. The onset of lathyrism was not delayed in our animals by the addition to

the diet of liberal amounts of liver extract. This observation is opposed to the findings of Díaz and Vivanco ('42) and Díaz, Vivanco and Mendoza ('43), who were able to prevent the development of toxicity in rats [8] fed *Lathyrus cicera* by feeding crude liver extract. Since their autoclaved extract failed to protect the animals, the presence of a new water-soluble thermostable protective factor in liver was postulated.

The results of our experiments with *Lathyrus odoratus* suggest that lathyrism is related to a thermostable toxic agent, which cannot be extracted from the seed by ether but which is removed readily by cold water or by 30% alcohol. Experiments with other species of *Lathyrus* support this observation.

The growth of the young animals fed other species of *Lathyrus*, which were not acutely toxic, is shown in table 3. The growth responses calculated per 100 gm of food for periods of 4 and 7 weeks respectively are presented for both the experimental groups (fed the *Lathyrus* peas) and their paired controls (fed the edible white pea). Since, in certain cases, definite failures in food consumption were observed and toxicity developed as the experiment progressed beyond 4 weeks, the gains in the 1st 4 weeks are perhaps more significant. The number of animals is the number of rats fed the experimental diets; the number of control rats (edible split pea diet) equalled them in all cases. The experiments with certain species were continued beyond 7 weeks; this is indicated in the column "Total period." The last column ("Clinical") presents the time required to develop symptoms of paralysis, lameness and similar indications of experimental lathyrism.

Lathyrus sativus

Contrary to observations in both man and many domestic animals, in whom lathyrism, both experimental and clinical, has been noted after the feeding of this species, we have ob-

[8] Díaz and his co-workers observed toxicity in rats fed *Lathyrus cicera*, but emphasized that the symptoms were not those of lathyrism as seen in either man or the horse.

TABLE 3 [1]

Growth of young, white rats fed various species of Lathyrus

LATHYRUS SPECIES	NUMBER OF RATS	GAINS PER 100 GM FOOD					CLINICAL NOTES
		4 weeks		7 weeks		Total period	
		Experimental group	Control group	Experimental group	Control group		
		gm	*gm*	*gm*	*gm*	*wks.*	
Sativus	6	21.6	23.9	19.4	21.2	14	None
Sativus	4	28.8	28.2	21.9	19.8	7	None
Cicera	6	22.1	22.4	20.8	20.1	7	None
Cicera (new lot)	3	19.2	22.3	15.9	17.1	21	None
Aphaca	5	14.9	30.4	18.8	30.4	17	Non-typical
Hirsutus	4	23.1	...	17.9	...	27–43	25, 27, —, 28 [2]
Hirsutus (new lot)	3	19.2	26.8	14.4	24.1	7–23	11, 10, 6
Tingitanus [3]	5	—2.5	6.8	3.6	7.5	12–33	25, 25, 12, 25, 33
Tingitanus (extracted)	3	25.7	25.1	14.6	17.8	12–14	None

[1] All the animals received diets containing 50% pea meal and 10% casein except the *L. tingitanus* group, to which 25% pea meal was fed. The figures in the column headed "Clinical notes" represent the period in weeks in which symptoms of marked paralysis appeared. The animals fed *L. tingitanus* showed the osseous malformations associated with lathyrism, but developed no marked paralysis.

[2] Died in 43 weeks.

[3] All animals of this group were either dead or killed at the period (weeks) indicated in the last column.

served satisfactory growth in rats fed this legume over periods of 7 to 14 weeks. In the first series of 6 rats (table 3), the food intake of the experimental group was limited to 10 to 12 gm daily, while in the 2nd series the voluntary food intake often reached 16 to 17 gm daily. The animals ate the *L. sativus* more eagerly than any other experimental or control diet. The gains per 100 gm of diet were essentially the same as those of the litter mates fed the white pea diet.

These results are in agreement with those of Díaz and Vivanco ('42), McCarrison and Krishman ('34), Visco ('24b) and Zagami ('31), who were unable to observe symptoms of lathyrism in young rats fed *L. sativus*. McCarrison and Krishman ('34), however, who fed *L. sativus* as the sole component of the diet, reported that the animals refused food after 6 weeks and died from asthenia. The animals of Díaz and Vivanco ('42), which received diets composed exclusively of raw or cooked *L. sativus*, also grew less rapidly than the controls on a mixed diet, although no deaths or signs of lathyrism were observed in 8 or 9 weeks. Zagami ('31) also observed retarded growth and failure of normal development, even though symptons of lathyrism were not present. Our animals fed *sativus* at the 50% level made good gains and continued to grow throughout a period of 14 weeks.

Lathyrus cicera

The ingestion of this species of *Lathyrus* by both man and domestic animals (Schuchardt, 1886–87; Stockman, '29) has led to the development of lathyrism, although it has not been possible to produce lathyrism by feeding *L. cicera* to rats, even when the diet is composed exclusively of this pea. Similar results with rats were obtained in our own experiments in which *L. cicera* was fed at the 50% level. The rats ate the diet well; good growth comparable to that of control animals fed the edible split pea of commerce was obtained; and no symptoms of lathyrism were noted (table 3). No spinal curvature or abnormalities of the long bones were seen at autopsy.

Lathyrus aphaca

None of the rats fed diets containing this pea developed any of the characteristic symptoms of lathyrism within a period of 17 weeks. Although no acute toxicity was apparent, the gains per 100 gm of food eaten were definitely less than those of the pair-fed control group which received the edible white pea diets (table 3). There appeared in several of the animals some alopecia, notably around the neck. The addition of inositol to the diet failed to influence the development of the alopecia in these animals. It is evident that the acute toxic factor associated with *L. odoratus* and some other species of *Lathyrus* to be discussed is not present in *L. aphaca,* but that the seed, presumably the protein of the seed, is inferior from the nutritive standpoint to *L. sativus, L. cicera,* or *Pisum sativum.*

Lathyrus hirsutus

In the 1st series of experiments with this species of *Lathyrus,* the animals grew moderately well for 13 or 14 weeks and up to that time showed no signs of paralysis or spinal curvature. Subsequently, however, they lost weight, developed paralysis and spinal curvature and died in 26 to 43 weeks. We have never observed so long a period of survival in rats fed a diet containing the sweet pea *(L. odoratus).* On the latter diet the rats developed symptoms of paralysis in 7 weeks or earlier and in no cases survived more than 16 weeks. We were able to secure a 2nd sample of *L. hirsutus* seeds grown in a different season, but in the same region. On a diet containing these peas, growth was poorer and the animals developed the characteristic symptoms of lathyrism in 6 to 11 weeks. It is evident that the seeds of *L. hirsutus* are toxic and produce symptoms similar to those occasioned by *L. odoratus,* although the toxicity is definitely less. Pathological changes in the long bones and spinal column similar to those produced by diets containing *L. odoratus* were reported by Dr. Carl V. Weller, to whom we are indebted for the pathological studies.

Lathyrus tingitanus

This seed, when fed at a level of 50% in the diet, proved very toxic. The animals ate the diet poorly and died in from 8 to 25 days. Thus rat 83 consumed only 20.5 gm of the mixed diet (equivalent to approximately 10 gm of the pea meal) over a period of 9 days and died on the 9th day. The addition of 100 mg of powdered liver extract [9] to the diet, while it resulted in some increase in food consumption, did not in any way decrease the toxicity. When *L. tingitanus* was incorporated into the diet at the lower level of 25%, the animals survived for longer periods of time but lost weight in the 4-week period and had made little recovery at the end of the 7-week period (table 3). Of a group of 5 animals fed at this level three died and two were killed at the end of 25 and 33 weeks respectively. All of the animals of the group developed spinal curvature and the other skeletal changes characteristic of lathyrism in the rat. Addition of powdered liver extract over a period of 15 weeks failed to improve the growth or to prevent the development of symptoms. When the rats were fed the seed extracted with 30% alcohol as described previously, the diet was eaten readily even at the 50% level and the animals made essentially the same gains as the controls (table 3). No signs of lathyrism were observed in 12 to 14 weeks. This is in marked contrast to the experiments with the unextracted seed in which, at the 50% level, the food consumption was poor, the animals lost weight rapidly, and died in from 8 to 25 days. It is evident that *Lathyrus tingitanus* contains a toxic substance, extractable by 30% alcohol, and that the extracted pea meal is of good biological quality as evidenced by gains per 100 gm of food consumed.

Lathyrus sphaericus [10]

This seed also proved to be highly toxic when fed at a level of 50% of the diet. The rats ate the diet poorly even when

[9] See footnote 3, page 540.

[10] These experiments were carried out with the cooperation of Prahlad C. Rajam.

powdered liver extract was added and young animals of 40 to 50 gm body weight died in 4 to 8 days. With slightly older and larger animals (85–95 gm), the period of survival (12, 14 23 and 38 days for one group) was longer, but the symptoms were similar. Even with fully grown rats of 275 to 300 gm in weight, death resulted in 25 to 45 days. The animals lost weight rapidly; this was in part due to low food consumption, but it should be pointed out that the loss in weight in the pair-fed controls was only about 50% of that of the experimental groups. The animals became weak and gradually lost control of their movements; the hind legs became stiff and later paralyzed. Since the young animals died after so short a period on the experimental diet, it was hardly to be anticipated that the skeletal changes seen in animals fed the seeds of *L. odoratus* or *hirsutus* would be observed.

Two rats of 115 to 125 gm body weight were fed the meal of *L. sphaericus,* previously extracted with 30% alcohol. The animals ate well and developed no symptoms of toxicity over a period of 21 days. Unfortunately, our limited supply of seed prevented further studies along this line.

Lathyrus sylvestris Wagneri

Of all the species of *Lathyrus* studied, this pea was the most toxic. When fed at the 50% level, the animals refused food within two to three days and died within 4 to 10 days. In many cases, symptoms of intoxication developed after the animal had eaten as little as 4 gm of the legume (8 gm of the mixed diet). The symptoms of intoxication were striking; the animals became hyperexcitable and during convulsive seizures would stand upright with head thrown back and with increased and spasmodic respiration. These seizures were repeated at frequent intervals and could be induced readily by external stimuli (touching animal with a rod, knocking on the cage, etc.). We observed the same symptoms of intoxication when seed grown in both Washington and Oregon and in different seasons was fed. With larger rats, the same picture was presented but the symptoms were somewhat delayed.

One rat of 250 gm survived the acute stages of toxicity and was fed the laboratory stock diet for over 8 months. However, it was possible to induce convulsive seizures by external stimuli even up to approximately 8 months after the diet containing *L. sylvestris* was withdrawn.

The same results were obtained when a diet containing 50% of finely pulverized *sylvestris* hay and 20% of casein, or the hay alone, was fed to larger rats. Characteristic symptoms and death resulted in from 6 to 13 days. In one experiment, the animal had consumed only 4 gm of hay prior to death.

The toxic agent could not be extracted from the pea meal by ether but could be removed by extraction with 30% alcohol. Gains of 11.7, 15.0 and 23.2 gm per 100 gm of food were obtained with the extracted pea meal, gains comparable to those of control animals under similar conditions of diet but receiving white peas.

The results of our studies with 8 species of *Lathyrus* have shown the presence of a factor in the seeds, with the exception of those of *L. sativus, L. cicera* and *L. aphaca,* which is toxic for rats under the conditions of our experiments. It is yet to be determined whether experiments carried out for longer periods of time would reveal the presence of a toxin for rats in the three apparently nontoxic species. It is notable that two of these species *(L. sativus* and *cicera)* have been commonly associated with human lathyrism. In those species in which we have undertaken to extract the intoxicant, the principle has been soluble in 30% alcohol and the residual meal after the alcohol extraction has been devoid of toxicity and as effective in the promotion of growth as the white split pea of commerce, when fed at the 50% level with 10% of casein.

Of the species studied, *Lathyrus sylvestris Wagneri* has proved most toxic. Its action suggests the presence of a very powerful neurotoxin. It is of interest to note here the careful description of the pathological anatomy of a human case of lathyrism *(Lathyrus sativus)* reported by Filimonoff

('26), in which extensive injury to the nervous system was observed. He also mentions many observations from the older Russian literature not available to us. Of particular significance is the citation (p. 79) of the experiments of Spirtoff with dogs in 1903, in which acute lathyrism with marked nervous injury was produced by prolonged feedings of *L. sativus, clymenum, cicera* and *sylvestris* in considerable amounts. Of these, *Lathyrus sylvestris* was most rapidly toxic and produced more severe injury to the gray matter of the cerebrum, notably the cortex, than did the other peas. The toxicity of the foliage of this species for certain domestic animals is recognized in our Western states (Grunder and Dickson, '48; Haag, '48). Further studies of the nutritive value of *Lathyrus* species are in progress in this laboratory.

SUMMARY

The nutritive values of the seeds of a number of species of *Lathyrus* of importance in human or animal nutrition when fed at a level of 50% with 10% of a biologically superior protein (casein) and with adequate minerals and vitamins have been studied in young white rats.

In confirmation of the studies of Geiger, Steenbock and Parsons ('33), nutritive failure and experimental lathyrism resulted when young white rats were fed diets containing ground sweet peas *(Lathyrus odoratus)*. Since the diets contained also 10% (in a few experiments, 25%) of casein, a protein or amino acid deficiency is not believed to be a causative factor.

The condition is believed to be associated with the presence of an intoxicant, which is stable to heat (24 hours at 80°, 6 hours at 120° in an autoclave) and which can be extracted readily by cold water or 30% alcohol. The extracted sweet pea meal was not toxic.

Although roentgenological and pathological observations indicated certain changes suggestive of scurvy, the addition of 12 mg of ascorbic acid daily to the diet failed to prevent lathyrism. The ascorbic acid content of the blood and tissues

of rats fed the sweet pea diet and the sweet pea diet plus ascorbic acid was the same as that of control animals of the same age fed the usual stock diet. When liver extract was added to the sweet pea diet, the development of lathyrism was neither prevented nor delayed.

When phytin was added to our control (white pea) diets, no toxicity was observed.

Lathyrus sativus and *cicera* showed no toxicity and promoted growth as effectively as did the edible white split pea of commerce. *Lathyrus aphaca* was nontoxic, but was inferior in growth promotion to the other species.

Lathyrus hirsutus, tingitanus, sphaericus, and *sylvestris Wagneri* were toxic, and the last three were unacceptable to the rats (they are listed in order of increasing toxicity). Of these, *Lathyrus sylvestris Wagneri* was most acutely toxic and appeared to contain a toxic substance which produced marked injury to the nervous system. The toxic material of *L. tingitanus, sphaericus* and *sylvestris Wagneri* could be extracted by 30% alcohol and the extracted meal was effective in promoting growth.

With the species less acutely toxic *(L. hirsutus* and *tingitanus),* it was possible to obtain the skeletal changes described as typical of lathyrism.

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STUDIES ON BERIBERI IN AN ENDEMIC SUB-TROPICAL AREA [1]

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(Received for publication June 18, 1948)

Beriberi ranks very high in the Philippines as a cause of illness and of death. During 1946 and 1947 it ranked second only to pulmonary tuberculosis as a cause of death. The respective mortality rates per 100,000 population for the two diseases were 148.21 and 170.06 in 1946. In 1947 beriberi claimed 24,296 lives with a mortality rate of 131.92 per 100,000, compared with 116.13 in 1939 and 111.87 in 1940. About two-thirds of the deaths are of infants.

While there are still many unknown factors in the etiology of beriberi, it has been commonly accepted that in rice-eating countries the disease is due, in a practical sense, to the consumption of large quantities of highly polished rice. Clinical beriberi is endemic in certain areas in the Philippines where rice is the chief staple food. Mortality from beriberi tends to be highest in those provinces where rice is grown most intensively.

[1] This study has been made possible by a grant from the Williams-Waterman Fund of Research Corporation, New York City, to the Department of Health, Republic of the Philippines. Some equipment and supplies were authorized from funds allocated to the U. S. Public Health Service Rehabilitation Program in the Philippines in connection with the Philippine Rehabilitation Act of 1946 of the 79th Congress of the United States.

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An attempt is projected to reduce the incidence of beriberi in an endemic area in the Philippines by trial of artificial enrichment of the entire rice supply. Bataan was selected because of its high beriberi mortality, the presence of only one national highway, whereon rice traffic may be controlled readily, and the generally uniform economic and agricultural conditions in all parts of the province. This paper deals with the findings of an 8-month clinical beriberi survey in that province, in contemplation of the introduction of enriched rice later in 1948. For comparison the survey will be repeated at a later date.

Metcoff and his co-workers ('45) described a nutritional survey at Norris Point, Newfoundland, in June 1944. The survey was done with a two-fold purpose; (1) rapid assessment of nutritional status; (2) establishment of a baseline for comparison with conditions after the then-contemplated introduction into Newfoundland of enriched flour. Our study thus has a pattern similar to that of Metcoff et al.

Of the 12 towns in Bataan, 9 were surveyed by sampling. From 11.11% to 64.04% (average 22.26%) of the total population in each town was examined clinically. The total number of persons examined was 12,384, among whom 1,580 cases of frank or suspected beriberi were encountered. The cases of beriberi were classified as frank or apparent, suggestive, and clinically doubtful. The population surveyed was classified as (a) infants; (b) children; (c) expectant mothers; (d) nursing mothers; (e) other adults.

MATERIALS AND METHODS

Three survey teams, each consisting of a full-time Medical Officer and a Clerk-Helper, were constituted and three municipalities were assigned to each team. Each physician made a thorough physical examination of 1,000 to 2,500 random subjects in each municipality assigned to him. Municipalities in the Philippines consist of a central town *(poblacion)* usually surrounded by several villages *(barrios)*. With few exceptions, the survey included a proportionate sample from

the central town and from each barrio. Two of 12 barrios in Hermosa were omitted because of disturbed socio-economic conditions there.

The physical examination included the taking of blood pressure, height and weight, and an examination for beriberi symptoms as indicated in table 3. The age, sex, occupation, address, family income per month, number of members of the family, other dependents, and similar pertinent data were recorded. Subjects who showed clinical beriberi, including clinically doubtful or suspicious cases, were referred to physicians on part-time service with the local units of the Bureau of Health. Clinical diagnosis of beriberi was accepted only when confirmed by the latter group of physicians.

Metcoff et al. ('45) classified nutritional deficiencies thus: (1) apparent, when the subject exhibited three signs or two signs and one or more symptoms characteristic of a dietary deficiency; (2) suggestive deficiency, when the subject had two signs or one sign and one or more symptoms; and (3) questionable deficiency if only one sign was present or if signs were less definite. In the present survey, the classification adopted by Metcoff was followed using the terms *frank or apparent beriberi, suggestive beriberi* and *clinically doubtful beriberi.*

Dann and Darby ('45) in discussing the zones of nutriture defined 5 zones or levels as follows: (1) saturated; (2) unsaturated, but functionally unimpaired; (3) potential deficiency disease; (4) latent deficiency disease, which was described by them as the mildest clinically-detectable form of deficiency characterized by "vague, indefinite, non-specific symptoms," and (5) clinically manifest deficiency disease which is divisible into mild or severe forms. Mild deficiency is that stage of the disease in which the classical syndrome has not yet been manifested but there are present highly suggestive signs and symptoms sometimes requiring laboratory confirmation and therapeutic tests to establish the diagnosis. The classical syndrome is present in severe deficiency. In this work, clinically doubtful beriberi falls under the classi-

fication of latent deficiency, and suggestive and frank beriberi are under the categories of mild and clinically manifest severe deficiency, respectively. Continuing, these authors re-stated the concept of the progressive development of deficiency diseases from a diminished concentration of the vitamins in the blood, succeeded by decreasing body stores, leading subsequently to a functional impairment, then to microscopic and finally to gross anatomic changes. In assessing nutriture they listed clinical examinations, demonstrations of a biochemical or physiological lesion, and estimates of the past dietary intake as the methods used. They emphasized, however, that estimation of the dietary intake is of secondary value at present, especially in potential or latent deficiencies. Similarly, Gordon ('47) divides the development of deficiency diseases into 4 stages: (1) suboptimal intake of one or several nutritional factors; (2) depletion of storage reserves; (3) appearance of a biochemical lesion detectable by chemical tests; and (4) development of tissue changes.

Darby and Milam ('45) in a field study of the prevalence of clinical manifestations of dietary inadequacy included in their survey a medical history, a physical examination, a series of laboratory determinations of blood specimens, and the recording of individual 7-day food intakes from which the average daily intake of the various nutrients was determined by the use of food tables. King ('48) noted that chemical methods of appraising nutritional status are being more widely used in order to furnish a better basis for diagnosing deficiencies, not by replacing but by supplementing dietary histories and clinical examinations.

In this survey the signs and symptoms of clinical beriberi were listed on a card on which the examining physician indicated for each person the presence or absence and the severity of existing signs and symptoms (see table 3). In drawing up the card of symptoms and signs, the descriptions by Jolliffe ('45), Albert and Abad ('47), Eddy and Dalldorf ('41), Youmans ('43), McLester ('43), Wohl ('45) and Hibbs ('46) were consulted.

FINDINGS AND OBSERVATIONS

The incidence of clinical beriberi in Bataan, in people of various ages and physiological conditions, is presented in tables 1 and 2, the findings being classified by degree of deficiency and place of residence. An analysis of the data

TABLE 1

Incidence of clinical beriberi in Bataan by age, physiological condition and degree of deficiency

POPULATION GROUP	FRANK BERIBERI			SUGGESTIVE BERIBERI			DOUBTFUL BERIBERI			TOTAL		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
	no.	%	%	no.	%	%	no.	%	%	no.	%	%
Infants (0–2 years)	5	0.48	.040	12	1.07	.097	16	1.56	.129	33	3.03	0.27
Children (2.01–15 years)	13	0.43	.105	7	0.28	.057	24	0.68	.194	44	1.58	0.356
Expectant mothers	43	6.65	.346	74	11.45	.597	122	18.88	.985	239	36.99	1.93
Nursing mothers	79	4.78	.636	120	7.27	.970	291	17.63	2.350	490	29.69	3.96
Other adults	105	1.82	.845	230	4.16	1.860	439	7.60	3.540	774	13.41	6.24
Total population	245	...	1.99	443	...	3.58	892	...	7.24	1580	....	12.76

(a) Number of cases found.

(b) Per cent incidence in group.

(c) Per cent incidence in population.

shows the following: (a) There is no significant difference in the incidence of clinical beriberi between the urban population ("poblacion") and the rural population ("barrio"); (b) among the groups specified in table 1, the incidence of clinical beriberi, with reference to the number of persons examined in each group, is highest in "expectant mothers," then in "nurs-

TABLE 2

Incidence of clinical beriberi in Bataan by degree of deficiency and place of residence

MUNICIPALITY	"POBLACION" (CENTER) OR "BARRIO" (VILLAGE)		FRANK BERIBERI		SUGGESTIVE BERIBERI		DOUBTFUL BERIBERI		TOTAL	
			Cases (a)	Incidence (b)	Cases (a)	Incidence (b)	Cases (a)	Incidence (b)	Cases (a)	Incidence (b)
			no.	%	no.	%	no.	%	no.	%
Hermosa	Poblacion	(22.4%)	9	1.69	17	3.19	57	10.71	83	15.60
(26.95%)	Barrios	(31.24%)	6	0.78	15	1.95	68	8.94	89	11.58
	Total		15	1.15	32	2.46	125	9.61	172	13.23
Orani	Poblacion	(11.39%)	2	1.88	1	.94	9	2.54	12	11.32
(15.88%)	Barrios	(16.31%)	14	.87	25	1.56	109	6.83	148	9.28
	Total		16	.94	26	1.53	118	6.94	160	9.41
Samal	Poblacion	(12.73%)	3	.33	14	1.56	65	7.29	82	9.21
(15.42%)	Barrios	(20.86%)	2	.27	11	1.52	53	7.36	66	9.16
	Total		5	0.37	25	1.55	118	7.32	148	9.18
Abucay	Poblacion	(25.16%)	16	1.26	44	3.49	80	6.32	140	11.07
(25.02%)	Barrios	(24.32%)	7	2.91	10	4.16	13	5.41	30	12.53
	Total		23	1.53	54	3.59	93	6.19	170	11.30
Balanga	Poblacion	(0%)	..	...	...	...	...	.....	...	.....
(11.11%)	Barrios	(11.11%)	24	1.52	121	7.93	225	14.75	370	24.26
	Total		24	1.52	121	7.93	225	14.75	370	24.26
Pilar	Poblacion	(88.16%)	9	2.27	12	3.03	22	5.56	43	10.88
(41.18%)	Barrios	(34.56%)	24	2.31	48	4.78	43	4.19	115	10.75
	Total		33	2.18	60	3.96	65	4.55	158	10.69
Orion	Poblacion	(13.78%)	34	3.95	42	4.88	31	3.60	107	12.44
(17.10%)	Barrios	(25.0%)	56	8.84	54	8.23	25	3.81	135	20.17
	Total		90	5.92	96	6.34	56	3.70	242	15.96
Bagac	Poblacion	(64.04%)	37	2.86	19	1.47	18	1.39	74	5.72
(64.04%)	Barrios	(0%)	..	...	..	...	..	...	..	...
	Total		37	2.86	19	1.47	18	1.39	74	5.72
Moron	Poblacion	(66.03%)	2	.21	9	.95	51	5.38	62	6.54
(61.37%)	Barrios	(44.09%)	0	0.00	1	.44	23	10.13	24	10.57
	Total		2	.16	10	.81	74	5.92	86	6.73
Total	All Poblaciones									
(9 out of	(21.13%)		112	2.26	158	3.19	333	6.72	603	12.17
12 towns)	All Barrios									
(22.26%)	(23.50%)		133	1.67	285	3.54	559	7.03	977	12.25
	Total Bataan		245	1.99	443	3.58	892	7.24	1580	12.76

(Numbers enclosed by parentheses indicate % of population examined.)

(a) Number of cases found in area.

(b) Per cent incidence in population of area.

ing mothers," "other adults," "infants," and "children"; (c) however, in relation to the total population of the community, the highest incidence of beriberi is in "other adults," followed by "nursing mothers," then by "expectant mothers," "children" and "infants" (the low incidence in infants compared with the high mortality is a reflection of the rapidity of fatal terminations in such cases); (d) the incidence of the various degrees of beriberi follows a uniform pattern of distribution among groups; (e) considering the total population surveyed by the criteria of dietary deficiencies given by Dann and Darby ('45) and by Gordon ('47), the incidence of clinical beriberi in all population groups totals 1.99% for frank beriberi, 3.58% for suggestive beriberi and 7.24% for doubtful beriberi, or 12.76% for all types; (f) analysis of the raw data demonstrates no influence of sex outside of the physiological conditions of pregnancy and lactation; (g) the incidence by towns or municipalities varies from 5.72% of the total population surveyed in Bagac to 24.26% in Balanga, with an average of 12.76% for the total population of Bataan. Frank beriberi is lowest in incidence at Moron with 0.16% and highest at Orion with 5.92%, with an average of 1.99%. Suggestive beriberi varies from 0.81% in Moron to 7.93% in Balanga, with an average of 3.58%. Clinically doubtful beriberi had an incidence ranging from 1.39% in Bagac to 14.75% in Balanga, with an average of 7.24%.

Frequency of signs and symptoms in clinical beriberi

The frequency of signs and symtoms by age, physiological condition, and degree of beriberi is shown in table 3. A separate column of additional symptoms has been provided for infantile beriberi. As is noted in the table, the most frequent symptoms in infants are aphonia, cyanosis, opisthotonus, and purposeless motions. In children, the most frequent signs and symptoms are referable to the nervous system; next, to the skin, muscles and cardio-vascular system. In expectant and nursing mothers the majority of the signs and symptoms

TABLE 3

Frequency of signs and symptoms in clinical beriberi in Bataan by age, physiological condition and degree of deficiency [1]

BODY REGION OR SYSTEM INVOLVED WITH SIGNS AND SYMPTOMS	INFANTS (0–2 YRS.) (33) [2]			CHILDREN (2.01–15 YRS.) (44)			EXPECTANT MOTHERS (239)			NURSING MOTHERS (490)			OTHER ADULTS 15 YEARS AND OVER (774)		
	F [1]	S	D	F	S	D	F	S	D	F	S	D	F	S	D
Mouth:															
Cyanosis	4	9		2			5	3		28	17		38	29	1
Numbness of lips							18	15	16	31	46	63	33	58	88
Tremor of tongue							1	1					5		
Gastrointestinal:															
Anorexia						1		6	2	6	9	10	7	12	27
Vomiting		2					2	1		9	2	1	6	2	
Constipation	1	1				1	4	8	11	8	21	30	17	22	49
Waist tightness					1		10	8	15	27	25	34	32	46	59
Cardiovascular:															
Fatigability				9	3	5	26	41	48	43	52	80	62	102	165
Palpitation				3		4	20	27	32	35	52	62	45	75	149
Tachycardia							12	4		9	5		14	21	1
Murmurs				1			2	1		4	2			1	
Enlarged heart				5	2		4	1		3			5	2	
Edema				1			21	32		7	11		20	33	1
Nervous system:															
Tingling				6	5	14	33	59	88	61	101	222	80	192	351
Numbness				14	7	18	37	58	97	66	113	254	89	203	398
Reflex changes				8	2		11	8		43	29		56	72	2
Paresthesia									6	6	5	11	7	12	26
Skin and muscles:															
Pallor		1		11	1		26	11		59	42		70	54	1
Tenderness (calf)				6	3	11	24	28	58	30	44	141	40	77	283
Cramps				8	6	11	38	56	93	60	68	175	76	151	216
Infantile beriberi:															
Ptosis															
Purposeless motions	3														
Opisthotonus	5														
Head drop	1														
Aphonia	2	10	16												

[1] F = frank beriberi; S = suggestive beriberi; D = clinically doubtful beriberi.

[2] Number of cases examined.

are referable to the cardio-vascular system, the nervous system, skin and muscles. In other adults, the signs and symtoms are more or less uniformly distributed over all the organ systems involved.

Deaths and mortality rates from beriberi in Bataan Province

To help in forming a baseline for future assessment of the influence of artificial enrichment of rice on beriberi mortality in Bataan Province, the deaths and mortality rates for this disease for the last semester and for the full year of 1947 are presented in table 4. Almost two-thirds of reported deaths from beriberi in the province of Bataan in the year 1947 occurred in the second semester coinciding with our survey. This is perhaps due in part to the fact that the people of Bataan and the Medical Officers of the Bureau of Health in that province became more aware of the manifestations of beriberi after the survey was started in that province. It is noteworthy that as is indicated in table 4 the greatest proportion of deaths occurred in infants. Infantile death rates were 140.1 per 100,000 population for the full year of 1947 and 91.4 per 100,000 population for the second semester, compared to total beriberi death rates of 166.5 and 107.7, respectively. No deaths of expectant mothers from beriberi were reported in the year 1947 and there were only 4 deaths of nursing mothers, three of which occurred in the second semester.

For purposes of comparison, the 1947 annual mortality rate for beriberi for the whole Philippines was 131.68 per 100,000 population and for the City of Manila, 83.75. The authors are of the opinion that official morbidity statistics are not complete in view of the frequent failure of hospitals and of private physicians to report cases of beriberi to the health authorities.

"Fasting hour" urinary thiamine analysis

Hou ('39) reviewed the literature describing several studies of the 24-hour excretion of thiamine in normal subjects. He

TABLE 4

Mortality from beriberi in Bataan, 1947 [1] *(figures in parentheses record deaths reported for period July 1 – December 31, 1947; other figures are for full year)*

MUNICIPALITY	ESTIMATED POPULATION	INFANTS 0–2 DEATHS	CHILDREN 2.01–15 DEATHS	EXPECTANT MOTHERS DEATHS	NURSING MOTHERS DEATHS	OTHER ADULTS DEATHS	TOTAL POPULATION	
							Deaths	Rate per 100,000
Abucay	9,552	14 (7)	1 (1)	0	2 (2)	1 (1)	18 (11)	167.5
Bagac	2,226	1 (1)	0	0	0	0	1 (1)	44.9
Balañga	14,349	31 (19)	2 (2)	0	1	2 (2)	36 (23)	250.8
Dinalupihan	13,591	14 (8)	1 (1)	0	0	1	16 (9)	117.7
Hermosa	9,207	29 (18)	4 (3)	0	0	2 (1)	35 (22)	380.1
Limay	4,906	2 (1)	0	0	0	0	2 (1)	40.7
Mariveles	5,491	4 (3)	0	0	1 (1)	0	5 (4)	91.05
Moron	3,107	7 (6)	0	0	0	0	7 (6)	225.2
Orani	11,739	12 (12)	1 (1)	0	0	1	14 (13)	119.2
Orion	10,372	12 (11)	0	0	0	6 (1)	18 (12)	173.5
Pilar	6,251	5 (2)	0	0	0	0	5 (2)	79.9
Samal	7,666	7 (2)	0	0	0	0	7 (2)	91.3
Total Bataan	98,457	138 (90)	9 (8)	0	4 (3)	13 (5)	164 (106)	166.5

[1] Data furnished by District Health Officer of Bataan.

and the authors he cited believe that thiamine excretion in the urine is dependent upon dietary intake. The figures for daily excretions in 24 hours range from 70 to 510 μg in normal subjects. In beriberi cases excretion may fall to zero. Some data published in 1939 showed 24-hour excretions in normal subjects ranging from 60 to 895 μg. Holt and Najjar ('43) believe that a 12-hour overnight fast should precede the collection for thiamine analysis of urine accumulated in the bladder during the 13th hour only. Hence the term "fasting hour excretion test." Johnson, Henderson, Robinson and Consolazio ('45) studied the comparative merits in field nutritional surveys of fasting specimens, random specimens and oral loading tests. They found that in 46 normal subjects with no previous load, the fasting hour excretion is 6 to 24 μg of thiamine, with a mean of 11 μg. They are of the opinion that fasting levels are more sensitive measures when other tests cannot be done and that the fasting rates of urinary excretion are more sensitive measures of vitamin intake in the previous three weeks than the 4-hour oral load tests. In this survey the "fasting hour" urinary excretion of thiamine after a 12-hour overnight fast was determined by the method proposed by Mickelsen, Condiff and Keys ('45). The results of the "fasting hour" urinary thiamine excretion test of beriberi cases in Bataan broken down with respect to age, physiological condition and degree of beriberi are listed in table 5. It will be noted that the excretion of thiamine in beriberi cases varies from 0 to 7.68 μg in children, from 0 to 8.34 μg in expectant mothers, from 0 to 8.51 μg in nursing mothers, and from 0.62 to 4.06 μg in other adults. The means for cases from all population groups were 2.85 μg for frank cases, 2.82 μg for suggestive beriberi, and 2.75 μg for doubtful beriberi.

In a study of the "fasting hour" urinary thiamine excretion of 65 apparently "normal" subjects, the excretion during the 13th hour ranged from 0.73 to 20.04 μg, with a grand mean of 5.7158 ± 3.725 μg.

[1] Data furnished by District Health Officer of Bataan.

The dietary intakes of these cases for a 9-day period were recorded by a procedure of keeping dietary records similar to that described in Bulletin 109 of the National Research Council ('43), and that of Phipard ('44). Under this procedure dietary histories are assessed in conjunction with the nutritional status of selected subjects. Although according to Kruse ('44) it was observed in his surveys that biochemical methods demonstrated, not the nutritional status,

TABLE 5

"Fasting hour" urinary thiamine excretion in micrograms of beriberi cases in Bataan by age, physiological condition and degree of beriberi [1]

POPULATION GROUP	FRANK BERIBERI		SUGGESTIVE BERIBERI		DOUBTFUL BERIBERI		TOTAL CASES	
	Number of cases	Mean thiamine excretion	Number of cases	Mean thiamine excretion	Number of cases	Mean thiamine excretion	Number of cases	Mean thiamine excretion
		μg		μg		μg		μg
Children [2] (2.01–15 years)	13	3.39	7	2.57	24	2.55	44	1.69
Expectant [3] mothers	43	4.27	74	3.14	122	2.53	239	2.64
Nursing [4] mothers	79	2.32	120	2.63	291	2.42	490	2.41
Other adults [5] (15.01 years and over)	105	2.64	230	2.66	439	2.88	774	2.61
Total beriberi cases examined	240	2.85	431	2.82	876	2.75	1,547	2.72

[1] Number of cases examined for thiamine not necessarily equal to number of beriberi cases.

[2] Minimum value in micrograms — 0.00 (D.B.). Maximum value in micrograms — 7.68 (F.B.). D.B. = doubtful beriberi and F.B. = frank beriberi.

[3] Minimum value in micrograms — 0.00 (F.B.). Maximum value in micrograms — 8.34 (F.B.). F.B. = frank beriberi.

[4] Minimum value in micrograms — 0.00 (F.B.). Maximum value in micrograms — 8.51 (S.B.). F.B. = frank beriberi and S.B. = suggestive beriberi.

[5] Minimum value in micrograms — 0.62 (F.B.). Maximum value in micrograms — 4.06 (D.B.). F. B. = frank beriberi and D.B. = doubtful beriberi.

but mainly the nature of the immediate past dietary intake, the data gathered from the dietary histories in this survey were considered in relation to thiamine excretion. The values for calories and for thiamine in the various articles of the diet were obtained from the food tables prepared by Concepcion ('47). The formula of Williams and Spies ('38) of micrograms of thiamine intake divided by calories and the formula of Cowgill ('39) on the thiamine requirements of individuals were used in eliminating 15 of the original 65 apparently "normal" individuals. Ten of the 15 subjects so eliminated have values of the T/Cal. ratio below 0.25 which, according to Williams and Spies, is indicative of clinical beriberi, and they also have thiamine intake deficits according to Cowgill's formula. Five of the 15 subjects showed stray figures. The grand mean of "fasting hour" urinary thiamine excretions after the foregoing procedure became 6.0828 ± 3.39 μg.

It becomes apparent from the foregoing discussion that the values obtained for the "fasting hour" urinary thiamine excretion in beriberi cases are not diagnostic of the deficiency when considered apart from the symptoms and signs characteristic of the disease. However, the presence of symptoms and signs together with low excretions of thiamine (from 0 to 2 μg) in the "fasting hour" urine specimen is helpful in the diagnosis of beriberi. The present authors see no correlation between the excretion of urinary thiamine in the "fasting hour" and the severity of beriberi. The writers of this paper believe that the dietary history, the physical examination and the "fasting hour" urine examination for thiamine should be taken together in the diagnosis of beriberi in individuals and in the assessment of beriberi incidence in a community. We are inclined to agree with Darby ('47) that "inasmuch as variability is a fundamental attribute in biology, it becomes impossible to fix a single figure as the requirement for a substance," and we may add that it is likewise impossible to say that a given figure for a biochemical test, as such tests are developed at present, is diagnostic of beriberi.

The incidence of clinical beriberi with respect to the thiamine-calorie ratio was studied more in detail by the computation of thiamine and calories in a 9-day dietary history of beriberi subjects selected at random in Bataan Province. The results of this study are shown in table 6.

There seems to be concentration of cases in the groups below Williams and Spies' ('38) T/Cal. borderline ratio of 0.25 in which 68% of all cases, 76% of frank cases and 80% of suggestive cases occur.

TABLE 6

Incidence of clinical beriberi with respect to thiamine-calorie (T/Cal.) ratio

T/CAL.	CASES NO.	FRANK		SUGGESTIVE		DOUBTFUL		MEAN FASTING HOUR EXCRETION
		no.	%	no.	%	no.	%	μg
0.10–0.149	7	1	14.28	3	42.85	3	42.85	2.71
0.15–0.199	34	7	20.58	7	20.58	20	58.82	3.65
0.20–0.249	43	5	11.62	10	23.24	28	65.11	2.41
0.25–0.299	21	2	9.52	2	9.52	17	80.95	3.17
0.30–0.349	10	1	10.00	3	30.00	6	60.00	1.87
0.35–0.399	6	0		0		6	100.00	2.50
0.40 up [1]	1	1	100.00	0		0		0.61

[1] Last item apparently a stray figure.

Rearrangement of the series according to an ascending order of deviation of actual thiamine intake from Cowgill's theoretical thiamine intake does not reflect any improvement on the above results as it involves only an internal shuffling of individual groups. In fact, 64.7% of all cases had no thiamine deficit, using Gowgill's formula.

Dietary practices of rice-eaters in Bataan

To complete the information given in this paper, inquiries were made about the dietary practices of rice-eaters in Bataan. It is noteworthy that 30% of the subjects studied, washed rice twice before cooking, 55% three times, and 15%

4 times. All rice washings were discarded by 81% of the subjects.

An impression of the dominance of rice in the prevailing dietaries may be gained from the following statements: Two-thirds of the families surveyed reported the use of meat only once a week; fish was consumed by 90% of the families 6 or 7 times a week; vegetables of some sort were used, by more than half the population, not more than twice a week.

SUMMARY

A clinical beriberi survey was undertaken in the Province of Bataan, Philippines, a sub-tropical area with endemic beriberi. The clinical survey included "fasting hour" urinary thiamine analysis. The population surveyed consisted of 11 to 64% of the total population in each of 9 of the 12 municipalities of the Province. A total of 12,384 persons were examined and 1,580 cases of frank or suspected beriberi were found, corresponding to 12.7% of the population. Frank beriberi occurred in 1.99%.

Beriberi is found in descending order of frequency in "expectant mothers," "nursing mothers," "other adults," "infants," and "children." However, the reported mortality from beriberi is highest in infants and then in "other adults," there being no deaths of expectant mothers and only a very small number among nursing mothers.

Discussion is presented of the frequency of signs and symptoms of clinical beriberi, of the limited usefulness of the so-called "fasting hour" urinary thiamine excretion test, and of the dietary practices of rice-eaters in Bataan.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the full cooperation, moral support and helpful advice given to this study by Brigadier General H. F. Smith, Assistant Surgeon General, U. S. Public Health Service, and Officer-in-Charge, Philippine Public Health Rehabilitation Program; the Honorable An-

tonio Villarama, Secretary of Health, Republic of the Philippines; Dr. Robert R. Williams, Chairman, Williams-Waterman Committee for the Combat of Dietary Diseases; Dr. Francisco Hernando, District Health Officer of Bataan Province and his Staff; and the various Government officials and the people of Bataan. The urinary thiamine analysis was done by the Misses Isabel Concepcion,[4] Araceli F. Guerrero,[4] and Dorotea Pesigan.[5] The progress and completion of this survey would not have been realized without the cooperation and assistance of the foregoing individuals.

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LIVER DAMAGE AND GROWTH IN THE RABBIT

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THREE FIGURES

(Received for publication May 28, 1948)

The rabbit is a potentially valuable experimental subject for nutritional research, particularly for the evaluation of forages and for the study of some minor-element deficiencies that plague the farm animal in many parts of the world. The use of the rabbit in such research has been hindered by our limited knowledge of its nutritive requirements. Our experience has been that rabbits suffer liver injury and subnormal growth both when fed a whole-milk-powder diet (fortified with iron, copper, manganese, and iodine) and when fed various purified diets. This report is a preliminary investigation into the cause and prevention of liver damage and subnormal growth in rabbits subsisting on seemingly adequate diets.

Rich and Hamilton ('40) observed liver cirrhosis in rabbits fed low-protein diets and reported that daily supplements of yeast prevented this damage. The protective factor in yeast was not identified, but the diets of the animals studied would suggest a deficiency of the labile methyl group. Blumberg, Mackenzie, and Seligson ('42) reported that the liver damage suffered by rabbits on a purified diet containing 10% casein was prevented by daily supplements of 300 mg of choline. Spellburg and Keeton ('40) reported fatty livers in rabbits and guinea pigs fed a scorbutigenic diet; they concluded that choline, lipocaic, or yeast is ineffective in preventing them. György ('44) suggested, in his review of experimental he-

patic injury, that a deficiency of the labile methyl group is not a complete answer to dietary-produced hepatic damage. Smith and Ellis ('47) observed fatty and cirrhotic livers in rabbits fed a whole-milk diet. Unpublished data (Smith, '44, '46) indicated that supplementation of the milk with members of the B complex, choline, yeast, or mineral mixtures containing Mg, Co, I, B, Cr, Pb, Zn, As, Mo, V, Se, Br, Be, Ni, Ti and F is not effective in preventing the liver damage.

Smith, Medlicott, and Ellis ('44) observed that the growth of rabbits fed a whole-milk diet supplemented with Fe, Cu, and Mn was only 60% of the growth of comparable rabbits fed a stock diet. Hogan and Hamilton ('42) reported that rabbits and guinea pigs grew normally on purified diets if supplemented with yeast or a water extract of dried liver, but that growth was subnormal on such diets supplemented with a vitamin mixture. Vivanco and Jimenez ('43) noted that the addition of fresh alfalfa enhanced growth and survival of rabbits fed a pea-flour and wheat-bran diet. Fresh grass has been reported essential in the feeding of breeding rabbits (Norfeldt, '42).

EXPERIMENTAL

The experimental animals used in the present study were Dutch rabbits bred in our stock colony. When the young were two weeks of age the stock diet in the cage was replaced with whole-milk powder fortified with a mineral mixture containing iron, copper, manganese, and iodine. The nursing dams were moved to another cage for several hours each day, where they were fed the stock diet. The restriction of the nursing litters to a supplement of whole-milk powder after they reached 14 days of age was instituted when it was observed (Smith, '44, '46) that the incidence of hepatic damage was reduced if the experimental animals had access to the stock ration in the pre-experimental period. The young were weaned at approximately 21 days of age, housed in individual wire-screened cages in a controlled-temperature room at 75°F., and fed the experimental diets ad libitum. All animals

were weighed weekly. At the completion of 70 days on the experimental regime, the surviving rabbits were sacrificed and the condition of the livers observed grossly. With the exception of the livers from rabbits on Diets 3, 4, 6, 7, and 11, all livers were sampled and at least one-half of these samples were examined microscopically. Formol fixed frozen sections, stained with Sudan IV and Sudan Black, were examined for fat infiltration. Sections fixed in Bouin's or Susa's fixatives and stained with hematoxylin and eosin (Mallory's or Masson's staining technique) were examined for extent of cellular damage and infiltration of connective tissue.

Four experiments were conducted at different times involving 23 different diets and 177 rabbits. Initially, 9 animals were assigned to a diet and, in subsequent trials, 6 to a diet. The composition of the experimental diets is shown in table 1. All supplements were incorporated into the diets and the diets stored at low temperatures (-15°C.). The aqueous and alcoholic extracts were made at room temperature with a proportion of one to 8 by weight of dehydrated alfalfa meal and water. The mixture was agitated for three hours, then allowed to stand overnight, and filtered in a Buchner funnel. The filtrate was concentrated under a vacuum with the flask containing the extract in a water bath heated to temperatures between 40° to 60°C. The residues were dried in an oven at 70°C. The animals receiving Diet 1 (whole-milk powder fortified with iron, copper, manganese, and iodine) served in each of the 4 experiments as the control group for both hepatic damage and growth. To facilitate discussion, Diet 1 is designated as the basal diet. The remaining diets may be divided into those primarily designed to study the liver damage (Diets 2 to 12 inclusive) and those primarily designed to study growth (Diets 13 to 23 inclusive).

RESULTS

Survival on all diets was good, with an overall mortality of less than 6%. More than half of the deaths occurred on the diets compounded of purified ingredients. In general,

TABLE 1

Percentage composition of diets [1]

DIET NO.	COMPOSITION
1	Milk [2] 100.
2	Milk 100, vitamin supplement.[3]
3	Milk 50, Ruffex [4] 15, starch [5] 33, minerals [6] 2.
4	Milk 50, Ruffex 15, dextrose 33, minerals 2.
5	Milk 90, alfalfa [7] 9.8, DL-methionine 0.2.
6	Ruffex 15, starch 40, crude casein [8] 20, fat [9] 11, yeast [10] 10, minerals 4.
7	Ruffex 15, dextrose 20, starch 20, crude casein 20, fat 11, yeast 10, minerals 4.
8	Ruffex 15, starch 40, sucrose 10, crude casein 20, fat 11, minerals 4, vitamin supplement.[11]
9	Ruffex 10, dextrin [12] 36, vitamin-free casein [13] 25, fat 25, minerals 4, vitamin supplement.[3]
10	Same as diet 9 plus cholesterol 0.15.
11	Milk 50, alfalfa 50.
12	Milk 75, alfalfa 25.
13	Milk 90, alfalfa 10.
14	Milk 95, alfalfa 5.
15	Milk 97, alfalfa 3.
16	Milk 99, alfalfa 1.
17	Milk 99.5, alfalfa 0.5.
18	Milk 97,[14] aqueous extract equivalent to alfalfa 10.
19	Milk 93,[14] residue aqueous extract equivalent to alfalfa 10.
20	Milk 99.7,[14] aqueous extract equivalent to alfalfa 1.
21	Milk 99.3,[14] residue aqueous extract equivalent to alfalfa 1.
22	Milk 99.8,[14] filtrate alcoholic precipitate of aqueous extract equivalent to alfalfa 5.
23	Milk 98.7,[14] alcoholic precipitate of aqueous extract equivalent to alfalfa 5.

[1] All diets were supplemented to contain a minimum of 10 mg Fe, 0.8 mg Cu, and 0.6 mg Mn.

[2] Klim, Borden Company.

[3] Milligrams/100 gm: choline 200, niacin 20, inositol 10, pyridoxine 0.7, thiamine 0.7, riboflavin 0.7, calcium pantothenate 1.5, biotin 0.25, folic acid 0.25, 2 methyl,3 phytl,1,4-4 naphthoquinone 0.075, alpha tocopherol acetate 7.5, crystalline A 0.20, viosterol 40.

[4] Fisher Scientific Company.

[5] Cooked starch.

[6] Osborne and Mendel.

[7] Seventeen per cent dehydrated alfalfa meal, W. J. Small Company, Kansas City, Missouri.

[8] Argentine crude casein.

[9] Primex, hydrogenated vegetable oil.

[10] Anheuser-Busch.

[11] Milligrams/100 gm: choline 200, niacin 20, inositol 10, pyridoxine 0.7, thiamine 0.7, riboflavin 0.7, calcium pantothenate 1.5, biotin 0.25, alpha tocopherol acetate 7.5.

[12] Merck N.F.V. white dextrin.

[13] Labco, Borden Company.

[14] Approximations.

the rabbits appeared healthy and thrifty, except those receiving Diets 9 and 10. On these two diets the rabbits were noticeably smaller, with rough, shaggy, dull coats and unthrifty appearances.

Liver injury

The essential observations on the condition of the livers of all rabbits surviving the 70-day experimental period are noted in table 2. The livers of the 24 surviving animals fed Diet 1 showed fatty and fibrous degeneration, characterized in the majority of cases by a reddish-yellow color and tenderness or slight resistance to probing: in some instances the liver was a light yellow in color, possessed a pebbled or "hob-nail" surface, and was turgid and hard. Histologically, the picture was one of early cirrhosis manifested by degeneration and destruction of hepatic cells, a regeneration of liver cells from those escaping destruction, a slight connective tissue proliferation at the portal canal, and an accompanying fatty infiltration of the liver cells (see figs. 1, 2, and 3, Plate 1). No ceriod pigment was evident. The histological studies will be presented in detail elsewhere.

The addition to the basal diet of 15% cellulose from rice bran,[1] 2% salt mixture, and 33% starch or dextrose (Diets 3 and 4) had no effect on the incidence of hepatic damage. The same result was noted when the basal diet was fortified with a "complete" vitamin mixture (Diet 2).

The livers of the animals receiving the diets compounded with purified ingredients using yeast as a source of the accessory food factors indicated slight fatty and fibrous degeneration in 9 of the rabbits, with the livers of 4 of the animals apparently normal (Diets 6 and 7). The feeding of a similar purified diet (Diet 8) with a vitamin mixture replacing the yeast resulted in all animals suffering liver damage.

Liver damage was also observed in rabbits receiving a diet formulated to approximate the composition of milk powder

[1] "Ruffex," Fisher Scientific Co., Pittsburgh, Pennsylvania.

TABLE 2

Summary of results pertaining to liver condition and average body weights and gains of rabbits

DIET NO.	DESCRIPTION	NO. OF ANIMALS		LIVER CONDITION	BODY WEIGHT		Gain [1]
		Initial	Final		Final	Initial	
					gm	*gm*	*gm*
1 [2]	Milk, 100%						
a		9	8	Fatty and fibrotic	800	189	611 ± 71
b		6	6	Fatty and fibrotic	914	246	668 ± 112
c		5	5	Fatty and fibrotic	820	268	552 ± 117
d		6	5	Fatty and fibrotic	909	210	699 ± 123
2	Milk 100% + vitamins	6	6	Fatty and fibrotic	956	211	745 ± 67
3	Milk 50%, Ruffex, starch	9	9	Fatty and fibrotic	748	238	510 ± 46
4	Milk 50%, Ruffex, dextrose	9	9	Fatty and fibrotic	637	208	429 ± 48
5	Milk 90%, alfalfa 9.8%, methionine 0.2%	6	6	Fatty and fibrotic	1073	293	780 ± 89
6	Purified + yeast 10%	9	7	5 fatty, 2 normal	893	230	663 ± 32
7	Purified + yeast 10%	9	6	4 fatty, 2 normal	867	202	665 ± 82
8	Purified + vitamin supplement	6	4	Fatty and fibrotic	815	266	549 ± 29
9	Purified + vitamin supplement	6	6	Fatty and fibrotic	561	172	389 ± 44
10	Diet 9 + cholesterol 0.15%	6	6	Fatty and fibrotic	487	190	298 ± 64
11	Milk 50%, alfalfa 50%	9	9	Normal	1181	242	939 ± 72
12	Milk 75%, alfalfa 25%	6	6	Fatty, fibrotic	1173	205	968 ± 91
13	Milk 90%, alfalfa 10%	6	6	Fatty, fibrotic	1350	252	1098 ± 66
14	Milk 95%, alfalfa 5%	6	6	Fatty, fibrotic	1176	217	959 ± 82
15	Milk 97%, alfalfa 3%	6	6	Fatty, fibrotic	1099	181	918 ± 82
16	Milk 99%, alfalfa 1%	6	5	Fatty, fibrotic	1259	225	1034 ± 61
17	Milk 99.5%, alfalfa 0.5%	6	6	Fatty, fibrotic	795	204	591 ± 85
18	Milk 97%, water extract equiv. to 10% alfalfa	6	6	Fatty, fibrotic	1037	288	749 ± 104
19	Milk 93%, residue water extract equiv. to 10% alfalfa	6	6	Fatty, fibrotic	1146	277	869 ± 153
20	Milk 99.7%, water extract equiv. to 1% alfalfa	6	6	Fatty, fibrotic	975	252	723 ± 95
21	Milk 99.3%, residue water extract equiv. to 1% alfalfa	6	6	Fatty, fibrotic	1114	255	859 ± 40
22	Milk 99.8%, alcoholic filtrate water extract equiv. to 5% alfalfa	6	6	Fatty, fibrotic	772	178	594 ± 76
23	Milk 98.7%, alcoholic precipitate water extract equiv. to 5% alfalfa	6	6	Fatty, fibrotic	1007	234	973 ± 58

[1] With standard errors.

[2] Statistical comparisons with respect to gains in body weight: **Highly significant ($P < 0.01$); *significant ($P < 0.05$). (For detailed discussion see text.):

Diet 1a with diets 3, 4, 6, 7, and 11**
Diet 1b with diets 8 and 13**
Diet 1c with diets 18 and 19
Diet 1d with diets 2, 9, 10, 12, 14, 15, 16*, 17, 20, 21, 22, and 23
Diet 8* with diet 9

in its content of protein, carbohydrate, and fat, and supplemented with a vitamin mixture (Diet 9). When 0.15% cholesterol was added to this diet (Diet 10), the severity of the hepatic damage was not affected.

When Diet 1 was supplemented with dehydrated alfalfa meal at the 50% level, liver damage was not observed (Diet 11). No protection from liver damage was afforded the animal if this level of supplementation with alfalfa meal was reduced to 10% (Diet 13) or to 25% (Diet 12).

Methionine (0.2%) exhibited no lipotropic action according to histological evidence when added to the basal diet containing approximately 10% dehydrated alfalfa (Diet 5).

Growth

In table 2, the average initial and final live weights and the average gain in body weight with its standard error are given for the surviving rabbits on all diets studied. Since the various diets were studied in 4 different experiments with a control group of animals receiving Diet 1 in each experiment, the average data for the control groups are given separately as Diet 1a, b, c, and d. Statistical comparisons were made only within experiments. As may be noted from the standard errors, the individual variation within groups, particularly those fed Diet 1, is large. Random breeding resulting in variable weaning weights and growth potentials was a contributing factor to the variation within the groups. The greater variability on the basal diet may be accounted for by the fact that severe liver damage occurred in one or two individuals in each of the groups on Diet 1. Apparently incipient liver damage does not affect growth under our experimental conditions, but the progression of this damage to a cirrhotic condition does influence growth. In the 24 surviving rabbits which received Diet 1, 6 were observed to to have suffered severe liver damages and all 6 showed either a decreased growth rate or a cessation of growth and a loss of body weight.

Although the obvious corrective action for large variability is to increase the number of animals, it was felt that in preliminary studies of this nature with a limited number of animals statistical precision could be sacrificed in favor of a more comprehensive survey and study of the nutritional abnormalities encountered in the rabbit subsisting on a diet of mineralized whole milk powder. Statistical significance was determined by the "t" value as calculated from the body weight differences and their standard errors. Odds greater than 19:1 are accepted as being significant, and odds greater than 99:1 are considered highly significant.

The addition of 50 and 10% supplements of alfalfa meal to the basal diet (Diets 11 and 13) resulted in a highly significant increase in body weight when compared to the gain in body weight of the respective control animals (Diets 1a and 1b), but only the 50% supplement prevented liver damage. When dehydrated alfalfa was fed at a level of 1% (Diet 16), a significant increase in body weight was observed when compared to the gains made by the rabbits receiving Diet 1d. The supplementation of the basal diet with alfalfa at levels of 25, 5, 3, and 0.5% (Diets 12, 14, 15, and 17) did not result in significantly increased gains as compared with gains made by the control animals (Diet 1d). If the 24 rabbits receiving the basal diet are treated statistically as a group (the average gain and its standard error would be 631 ± 49), all supplements of alfalfa meal with the exception of Diet 17 (0.5% alfalfa) resulted in a highly significant increased gain in body weight.

The feeding of water extracts and their residues as supplements to the basal diet at levels equivalent to 10 and 1% alfalfa (Diets 18, 19, 20, and 21) did not significantly stimulate growth when compared with the growth obtained in the respective control groups (Diets 1c and d). The supplementation of the basal diet with the filtrate and residue of an 80% alcoholic precipitation of an aqueous extract of alfalfa at a level equivalent to 5% alfalfa (Diets 22 and 23) did not

result in a body gain significantly greater than that of the control group (Diet 1d).

The significantly greater gain in weight of the animals fed Diet 8 compared to the body gain of the rabbits fed Diet 9 is of interest. Both diets were formulated from purified ingredients and supplemented with vitamin mixtures.

DISCUSSION

The results presented here (Diets 2, 5) indicate that the hepatic damage suffered by rabbits fed solely on a mineralized milk diet is not caused by a deficiency in this diet of the known members of the vitamin B complex, of the fat-soluble vitamins, or of the labile methyl group. That the liver damage is not caused by lack of roughage or by toxic constituents in the milk is indicated by a comparison of Diets 3, 4, and 11. In each of these diets milk made up to 50% of the mixture, yet the animals fed on Diets 3 and 4 suffered liver injury whereas the livers of the rabbits receiving Diet 11 were normal. Several authors have reported that liver damage may be produced in the rabbit by feeding cholesterol (Chalatow, '14; Baily, '16). However, the inclusion of cholesterol in a purified diet at a level comparable to the cholesterol concentration in whole-milk powder failed in the present case to increase the severity of the liver damage.

According to our results, then, dehydrated alfalfa seemingly contains an unidentified liver-protecting substance, but this protective substance seems to be present in relatively low concentrations since a 10 or 25% "alfalfa-milk" diet does not prevent liver damage.

We have also observed that the inclusion of 10% yeast [2] in purified diets partially protected the rabbit from liver damage. Rich and Hamilton ('40) reported that 5 gm of brewers' yeast fed daily as a supplement to a low-protein diet prevented the development of cirrhotic livers in rabbits. Spellburg and Keeton ('40) found that guinea pigs and rab-

[2] Anheuser-Busch.

bits suffered fatty degeneration of the liver on 30% whole-milk diets, but that yeast [3] was not protective at the 1% level.

The observation (Smith, '44–'46) that if the experimental animal was permitted to consume stock ration before weaning, liver damage was not invariably produced on the milk diet suggests that either (1) the animal was able to store sufficient quantities of the factor before being placed on the milk diet, or (2) a bacterial flora favorable for the production of the factor was well established in the brief pre-weaning consumption of stock ration. In our studies, the addition of dextrose or starch to the milk diet had no effect on the observed liver damage, nor did the formulation of purified diets with various carbohydrates.

Also of interest in connection with the problem of liver damage, produced by dietary means, which does not respond to the recognized lipotropic factors are the reports of Schwarz ('44a, '44b). This author observed liver damage in rats fed purified diets containing alkaline-washed casein which could be prevented by supplements of wheat germ oil, cream, sunflower seed oil, total lipids of adrenal gland, xanthine, or a 20-fold increase of alpha tocopherol over the normal requirement. Other workers (Forbes, '39) have reported that xanthine and xanthine-containing preparations prevent liver damage in rats subjected to carbon tetra-chloride poisoning.

The increase in body weight of young rabbits fed a basal milk diet is markedly stimulated with supplements of dehydrated alfalfa meal at a level as low as 1%. Kohler, Elvehjem and Hart ('36, '37) report increased growth in rats fed a "winter milk" diet supplemented with fresh grass, dried grass, or grass juice. These workers have also shown that dehydrated grass or grass juice is essential for the nutrition of the guinea pig fed a "winter milk" ration (Kohler, Elvehjem, and Hart, '38; Kohler, Randle, Elvehjem, and Hart, '39). It seems possible that the growth factor in dehydrated grass and grass juice essential in the nutrition of the rat and guinea pig is also necessary in the nutrition of the rabbit. Our fail-

[3] Fleischman's.

ure to extract with water the growth factor from dehydrated alfalfa in appreciable quantities is in agreement with the findings of Kohler, Randle, and Wagner ('39), who reported that the "grass juice factor" could not be extracted from dehydrated grass with water.

The differences in the growth-promoting properties of the purified diets varying in their contents of protein, carbohydrates, fat, roughage, vitamin supplements, and source of casein suggest the existence of another nutritional factor required by the rabbit. Since animals receiving the diet furnishing more protein and energy per gm of food and larger vitamin supplements gained the least in body weight, it would seem unlikely that the differences in composition and in vitamin supplements account for the differences in body gains. The protein of the diet associated with the larger gain in body weight was crude casein, while the protein of the other diet was a purified vitamin-free casein. This suggests that crude casein contains a factor essential for the nutrition of the rabbit. This postulated rabbit-growth factor in crude casein may be similar to strepogenin, which is reported to be a growth factor for the guinea pig (Wooley and Sprince, '45), and for which crude casein is a more potent source than purified casein; or, more likely, it may be similar to the animal protein factor said to be essential for normal growth in the rat (Cary et al., '46) and in the chick (Coombs, '47).

SUMMARY

A diet of whole-milk powder (fortified with manganese, copper, iron, and iodine) is inadequate for the rabbit in at least two respects. Growth is subnormal and the livers are fatty and cirrhotic. Large amounts (50%) of dehydrated alfalfa meal are needed to prevent liver injury, while only small amounts (1%) are needed to stimulate the growth rate. Experiments involving 23 diets and 177 animals indicate that milk does not contain a toxic factor responsible for these results, and that the results are not due to the lack of rough-

age or of any of the known fat- and water-soluble vitamins or of the labile methyl groups. Preliminary attempts to extract the growth-promoting factor from alfalfa with water were unsuccessful.

Purified diets when supplemented with a complete vitamin mixture offered no protection against liver damage, but when supplemented with yeast offered partial protection. In these diets crude casein produced better growth than purified casein, indicating the need of the rabbit for a third factor, possibly similar to the "animal-protein factor" of Cary et al. ('46) and Coombs ('47).

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PLATE 1

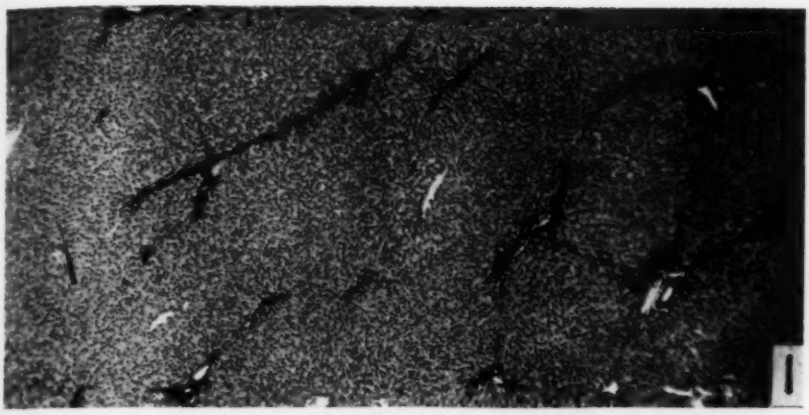
EXPLANATION OF FIGURES

Photomicrographs of damaged liver sections fixed in Susa's fixative, sectioned, and stained with hematoxylin and eosin.

1 This picture shows the slight proliferation of connective tissue originating at the portal canal as usually seen in the damaged livers (apprix. 60 ×).

2 The light areas indicate hepatic cell destruction accompanied by the infiltration of connective tissue (approx. 120 ×).

3 The vacuolated appearance of these hepatic cells indicates the areas from which fat was removed in the preparation of the tissue for sectioning and staining (approx. 600 ×).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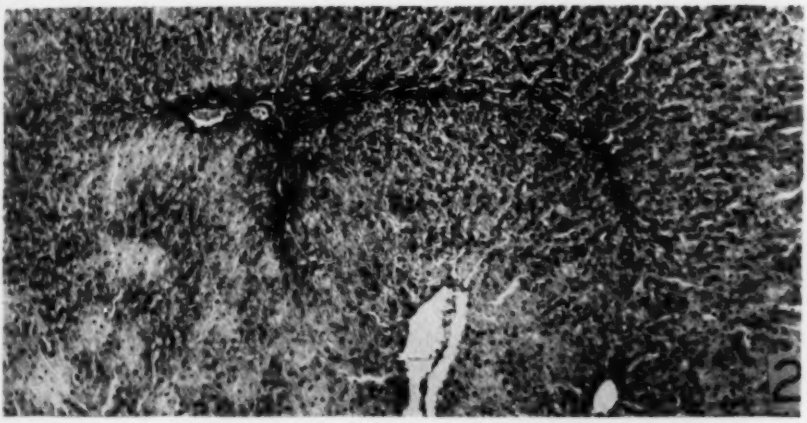
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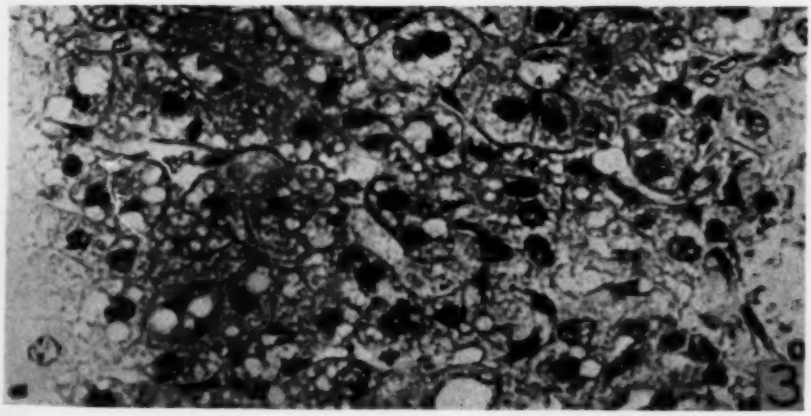
3

THE RELATIVE BIOLOGICAL VALUES OF THE PROTEINS IN CEREAL GRAINS[1]

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(Received for publication May 29, 1948)

Mitchell ('24a) found that on the same level of protein intake (5%) the proteins in whole brown rice are superior to those in corn and oats. Recently, while engaged in studies of the influence on growth and protein utilization of additions of small amounts of cultured food yeasts to the proteins in cereal flours and cereal grains, it was observed that, on the same protein level in the rations (5.8%) the proteins in polished rice are far superior to those in enriched milled wheat flour (Sure, '46, '47), as evidenced from gains in weight per gm of protein intake.

The purpose of this investigation was to determine the relative biological values of the proteins in rice, wheat, rye and corn, in both the milled and unmilled states[2]. Because of the low nitrogen content of rice, it was necessary to feed these cereal grains in such proportions as to introduce into the rations a total of not more than 5% of proteins.

[1] Research paper No. 863 Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

[2] The whole wheat, whole rye, and milled rye were furnished by General Mills, Inc., Minneapolis, Minn. The whole corn and milled corn (white table meal) were furnished by the Staley Manufacturing Co., Decatur, Ill. The whole brown and milled or polished rice was obtained from the Walton Rice Mills, Stuttgart, Ark. The milled wheat was Gold Medal (General Mills, Inc.) enriched flour, and the rolled oats were a Quaker Oats oatmeal product.

The biological values of the proteins of the cereal grains were determined by the nitrogen balance method (Mitchell, 24b, '44), in which the values are generally expressed as the per cent of absorbed nitrogen retained by the animal. Wistar strain albino rats were used and in all groups the sexes were equally divided. The ration fed was a modification of the one suggested by Mitchell and Hamilton,[3] which had the following percentage composition: Cellu flour, 2; lard, 10; Sure's salts no. 1 (Sure, '41), 4; cod liver oil, 1.5; wheat germ oil, 0.5; enough cereal grains to furnish 5% protein; and the balance, cerelose. The following components of the vitamin B complex were administered daily to each animal separately from the ration: 50 μg each of thiamine, riboflavin, pyridoxine, and nicotinic acid; 300 μg calcium pantothenate; 9 mg choline chloride; 6 mg *p*-aminobenzoic acid, and 2 mg inositol.

The nitrogen metabolism experiments were generally conducted on 24 animals, 12 males and 12 females, so that using 8 animals for each group, it was possible to study three cereal grains at one time. However, occasionally the results on one or two rats deviated so much from the rest of the group that it was necessary to eliminate such data from the averages; hence, data in some cases are shown for 6 animals. In the case of the rice experiments, it was necessary to use more animals in order to obtain reliable average figures.

Urinary and fecal balances were carried out for 7 days on an egg standardizing ration and 7 days on the experimental rations containing the various cereal grains. In each case the animals were allowed to get accustomed to consuming the entire rations for a pre-test period of from three to 5 days before the beginning of the nitrogen balance studies. The percentage composition of the egg ration is as follows: Dried defatted whole egg, 5.8; cellu flour, 2.0; Sure's salts no. 1, 4; cod liver oil, 1.5; wheat germ oil, 0.5; lard, 10.0; and cerelose, 76.2. The dried whole eggs were defatted by repeated extractions with large volumes of petroleum

[3] Personal communication.

ether at room temperature. The defatted dried whole eggs contained 69.2% protein and furnished 4.0% protein in the ration. Feces markers were used. The fiber contents of the diets were not equalized. However, the slight errors produced in measurements of fecal nitrogen excretions by neglect to balance the fiber contents of the standardizing diet and the test diets would be approximately the same for all the milled cereal grains and all the whole cereal grains, and would not influence findings as to the comparative biological values of the milled and unmilled cereal grains fed at the 5% protein level.

Our findings are summarized in table 1. It is evident from this table that when the milled cereal grains are fed as the only sources of proteins in the ration, at a 5% level of protein intake, the milled corn (as sold in grocery stores under the name of "table meal") is by far the poorest source and the milled rice is the best. There are no marked differences in the protein values of the milled wheat and milled rye. The net protein utilization values of the whole cereal grains are very similar. It will be noted that although the animals which received the whole corn as the only source of protein in the ration consumed about 40% less food than those which were fed the milled table meal, they utilized their proteins over twice as well. It would appear, then, that from the protein standpoint, the corn receives too drastic treatment in the course of being milled. Apparently too many bran layers, which carry lysine and tryptophane, the amino acids most deficient in the corn kernel (Mitchell and Smuts, '32), are removed. Recently it was demonstrated that the proteins in table corn meal are materially improved by the addition of such amino acids (Sure, '48).

As a control experiment, the biological value of lactalbumin,[4] was determined at a 5% protein level. The true digestibility was found to be 100%, and the biological value, 93.0%; therefore, the net utilization was also 93.0.

[4] Borden.

TABLE 1

The relative biological values of the proteins in cereal grains fed at a 5% level of protein intake

CEREAL GRAIN OR FLOUR	BODY WEIGHT	FOOD INTAKE	N [1] INTAKE	FECAL N	METABOLIC N IN FECES	FOOD N IN FECES	ABSORBED N	N IN URINE	METABOLIC N IN URINE	FOOD N IN URINE	FOOD N RETAINED	TRUE DIGESTIBILITY	BIOLOGICAL VALUE [2]	NET UTILIZATION [3]
	gm	*gm*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	%			
Milled rice (14)[4]	59.9	7.4	63.2	11.4	8.3	3.1	60.1	26.6	14.1	12.5	47.6	95.1	79.0	75.1
Milled wheat (8)	53.3	6.9	59.5	10.4	7.4	3.0	56.5	32.4	11.7	20.7	35.8	94.5	63.5	60.0
Milled rye (8)	62.6	7.6	65.3	14.0	8.1	5.9	59.4	33.1	15.1	18.0	41.4	90.5	69.7	63.1
Milled corn (8)	83.0	6.2	38.1	11.6	7.2	4.4	33.7	42.1	16.2	25.9	12.2	88.5	36.2	32.0
Whole rice (14)	59.4	4.8	44.2	7.6	5.0	2.6	41.6	21.3	15.1	6.2	35.4	94.1	85.1	80.0
Whole wheat (8)	60.2	5.0	43.6	9.5	5.9	3.6	40.0	23.9	17.1	6.8	33.2	91.7	83.0	76.1
Whole rye (6)	55.3	7.8	66.8	14.8	6.7	6.1	60.7	29.0	17.1	11.9	48.8	91.0	80.4	73.2
Whole corn (8)	53.7	3.9	30.9	6.5	4.4	2.1	28.8	19.7	15.1	4.6	24.4	93.0	84.7	78.8
Rolled oats (6)	60.5	7.6	65.4	11.8	6.6	5.2	60.2	27.3	16.5	10.8	49.4	92.1	82.1	75.6

[1] N = Nitrogen.

[2] Per cent of absorbed nitrogen retained in the body.

[3] The value for the true coefficient of digestibility multiplied by the biological value, divided by 100.

[4] Numbers in parenthesis indicate the number of animals used in the nitrogen balance experiment.

The results submitted in this paper are in harmony with the observations of Osborne and Mendel ('20), who reported that there were no notable differences in growth of albino rats when the entire oat, rye, and wheat kernels were used as the only sources of protein in their rations; and are also in agreement with the recent findings of Jones, Caldwell, and Widness ('48), who found from growth experiments that at the low 4.5% protein level, corn, hard wheat, soft wheat, barley and rice in the milled state showed essentially the same values The higher biological values for whole or brown rice than for milled or white rice are in agreement with the results of Kik ('32) but not in accord with those of Jones and associates ('48). As the protein level is decreased, the efficiency of protein utilization is increased; hence our results on biological values for the proteins in all the whole cereal grains are higher than are reported in the literature concerning corn, wheat, and rye, which have been studied for 8 and 10% levels.

Since corn is used widely as a source not only of calories but also of protein and vitamins by people of low income levels in the South, it would seem that there is a need for undermilling of corn, at least to the extent practised with wheat, rice, and rye, so as to leave in the milled product proteins of considerably higher biological value.

SUMMARY

The biological values of milled and whole cereal grains, fed at a 5% protein level, were determined by the Mitchell ('24b, '44) nitrogen balance method. The protein utilization for the milled cereal grains was as follows: Rice, 75.1; wheat, 60.0; rye, 63.1; and corn, 32.0. The findings for the whole cereal grains were: Rice, 80.0; wheat, 76.1; rye, 73.2; corn, 78.8; and rolled oats (oatmeal), 75.6.

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PTEROYLGLUTAMIC ACID REQUIREMENT OF THE RAT AND A CHARACTERISTIC LESION OBSERVED IN THE SPLEEN OF THE DEFICIENT ANIMAL[1,2]

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TWO FIGURES

(Received for publication June 12, 1948)

The deficiency syndrome in young rats caused by lack of pteroylglutamic acid (PGA) was first described by Black, McKibbin and Elvehjem ('41). Later, Spicer, Daft, Sebrell and Ashburn ('42) made a detailed study of this deficiency, showing that it is characterized not only by a cessation of growth but also by leukopenia, granulocytopenia, bone marrow hypoplasia and, occasionally, anemia. These same investigators observed that the blood dyscrasia and cessation of growth can be largely prevented or successfully treated with whole dried liver extract or with certain fractions of liver extract. Today, these substances are known to contain appreciable amounts of PGA.

In order to induce PGA deficiency in the rat, it is necessary to feed succinylsulfathiazole (SST) or some other inhibitor

[1] Published with the approval of the Director of the School of Tropical Medicine and the Director of the Agricultural Experiment Station of the University of Puerto Rico. A cooperative project.

[2] A preliminary report of this investigation was presented by the author at the meeting of the American Federation of Biological Societies held at Atlantic City, March 15–19, 1948.

together with the deficient diet, thus depriving the animal of the PGA that is known to be synthesized by its normal intestinal microflora.

The present paper is concerned with the determination of the pteroylglutamic acid level necessary to restore normal growth, white cell count, and spleen morphology to PGA-depleted rats.

EXPERIMENTAL

One hundred and fifty-three 21-day-old Wistar albino rats from our colony were kept in cages with raised wire floors, three to a cage. The basal purified diet free of PGA fed ad libitum to these animals had the following composition per kg: sucrose, 599 gm; vitamin-free casein,[3] 180 gm; salt mixture,[4] 40 gm; cellu flour, 40 gm; hydrogenated vegetable oil,[5] 100 gm; corn oil,[6] 20 gm (containing 52,000 I.U. vitamin A, 13,000 I.U. vitamin D, and 32 mg tocopherol); succinylsulfathiazole, 20 gm; choline chloride, 1 gm; inositol, 8 mg; nicotinic acid, 40 mg; calcium pantothenate, 44 mg; thiamine hydrochloride, 8 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 8 mg; *p*-aminobenzoic acid, 4 mg; biotin, 1 mg;[7] 2-methyl-1,4-napthoquinone, 10 mg.

As far as we have been able to determine,[8] our rat colony is free from *Bartonella* parasites.

[3] General Biochemicals, Inc.

[4] Sodium chloride, 4.35%; magnesium sulfate, 13.70%; sodium biphosphate, 8.72%; potassium phosphate (K_2HPO_4), 23.98%; calcium biphosphate $CaH_4(PO_4)_2H_2O$, 13.58%; ferric citrate (U.S.P. reagent, 17.5%, Fe), 2.97%; calcium lactate, 32.70%.

[5] Spry.

[6] Mazola.

[7] Provided by Dr. Elmer L. Sevringhaus of Hoffman-La Roche, Inc., Nutley, N. J.

[8] Smears of the blood of several animals, taken at random, were made at different intervals after splenectomy. In not a single case under observation were *Bartonella* parasites found in the blood. These animals were kept for several months and all of them gained weight at a normal rate. Their hemoglobin and red cell values were always within normal limits. Our thanks to Prof. William H. Taliaferro for examining the blood smears.

RESULTS

Induction of deficiency

The 21-day-old animals gained weight at a diminishing rate for three consecutive weeks and finally, between the third and 4th weeks on the basal diet, ceased growing altogether. Up to the third week all animals survived, but by the end of the 4th week only 91.2% of the initial number remained alive. At this time the average white cell count of the surviving animals was 3,635 cells per mm^3, and all the rats began suffering from alopecia with an average rating of 2.7.[9] Brownish cake formations on the ears, nose, and paws, as well as very soft feces, were characteristic conditions observed in practically all the depleted animals.

The average initial weight (21 days old) of the whole group of rats used was 32 gm (the group was composed of 81 males and 72 females), while the average weight of the 91.2% which survived the 4-week depletion period was 59 gm.

When autopsy was performed on the 24 animals that died between the third and 4th weeks, the only gross pathology observed in all was a yellowish distended intestine; infarcts appeared in the spleen of 73.5%.

Responses to different levels of pteroylglutamic acid

Every depleted animal was placed in an individual cage, after which 6 groups were formed of approximately the same numbers of males and females. One group did not receive the PGA supplement and served as a negative control group, while the other 5 were given, 6 times per week, in a separate dish, different levels of PGA in the form of its sodium salt.

The supplementation period lasted 5 weeks; that is, from the time the animals were 7 weeks old, when they were fully depleted, to their 12th week of growth. Weekly records were kept of their weights and of the food consumed. Before the

[9] We used the following alopecia rating: Normal — 1; mild alopecia — 2; severe alopecia — 3; total alopecia — 4.

supplement was started, white cell counts were performed in all. These counts were repeated at the end of the 5-week experimental period or as soon as an animal showed signs of impending death. Autopsies were performed in all animals. Again, the only gross pathology consistently observed in the negative controls, and in the rats receiving suboptimal levels of pteroylglutamic acid, was the presence of splenic infarcts and of a distended intestine of a yellowish color. Records

TABLE 1

Effects of various levels of pteroylglutamic acid (PGA) on depleted rats (5-week supplementation period)

PGA SUPPLEMENT	NUMBER OF ANIMALS	AVERAGE TIME OF SURVIVAL	AVERAGE GAIN OR LOSS IN WEIGHT	AVERAGE BASAL DIET CONSUMED PER ANIMAL PER DAY	AVERAGE GAIN OR LOSS IN LEUKOCYTES	AVERAGE INCIDENCE OF INFARCTS IN THE SPLEEN	AVERAGE DEGREE OF ALOPECIA AT DEATH [1]
µg		*days*	*gm*	*gm*	*cells/mm³*	%	
None (negative) (controls)	56	5.0	— 9.0	1.6	— 1755	81.8	3.0
0.25	13	25.2	+ 21.0	4.0	— 457	50.0	3.3
0.50	17	33.0	+ 28.0	4.0	+ 72	66.0	2.4
1.25	20	29.7	+ 53.0	4.5	+ 1710	5.0	1.6
5.00	16	34.5	+ 79.0	6.8	+ 3955	0	1.5
20.00	7	35.0	+ 106.0	8.5	+ 7050	0	1.0

[1] Alopecia rating: normal = 1; mild alopecia = 2; severe = 3; total = 4.

were kept of the degrees of alopecia observed in the different groups of animals. In table 1 we have summarized the results of this experiment, and figure 1 shows the corresponding growth curves.

The negative controls lived for an average of 5 days, losing only 9 gm of weight in that time. Prior to death, their average white cell count was 1,880 cells per mm³, that is, 1,755 cells less than at the start of the supplementation period. The inci-

dence of infarcts in the spleen in the negative controls was 81.8%. All of them showed a severe alopecia.

Although the group receiving 0.25 μg of PGA lost a considerable number of white cells during the supplementation period, they did show some positive growth. They also had a high incidence of infarcts in the spleen and their alopecia index was higher than that of the negative controls, probably owing to the fact that they survived for a longer period of time and could thus develop a more severe alopecia.

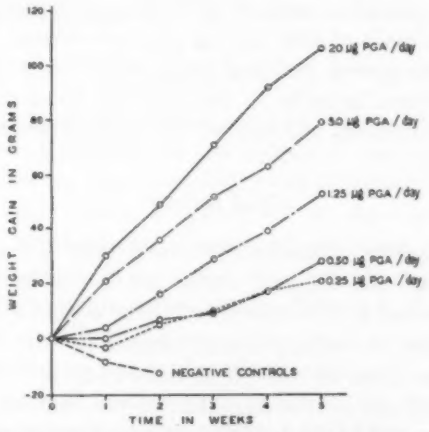

Fig. 1 Growth curves of PGA-depleted rats which received different levels of PGA 6 times per week for a period of 5 weeks.

The animals which received 0.5 μg of PGA made better gains in weight than those on 0.25 μg and gained few leukocytes during the supplementation period. The incidence of infarcts in the spleen was higher in this last group. On the other hand, the period of survival lasted 33 days while those on 0.25 μg of PGA survived for 25.2 days only.

The next group, on 1.25 μg PGA, gained 53 gm during the 5-week supplementation period — a better, but still subnormal, gain. However, they exhibited a very significant decrease in the incidence of infarcts; only one animal out of the 20

which composed the group, or 5%, showed this pathology. This level of PGA induced some tangible regeneration of the white cells, but at a subnormal rate. For this reason, at the end of the supplementation period, these animals were still suffering from leukopenia, as their average white cell count of only 5,345 cells per mm^3 showed.

The next group received 5 μg of PGA and were found, according to our standards, in a normal condition at the end of the 5-week supplementation period. Their weight gains as well as their white cell counts were within the lower limits of what we consider normal growth and normal leukocyte counts for animals of that age in our rat colony. None of the animals in this group suffered from infarcts in the spleen.

As can be seen in table 1, the rats fed 20 μg of PGA were completely normal at the end of the supplementation period.

Food intake

As the negative controls consumed only 1.6 gm of basal ration per day, the thought could not be discarded that the infarcts observed in the spleens of these animals might be the result of some secondary lack, induced by the small intake of food rather than by the PGA deficiency *per se*. This was partially ruled out by the fact that in the case of the animals on 0.25, 0.5 and 1.25 μg of PGA the incidence of infarcts in the first two groups was above 50%, while in the last group it was only 5% even though the intake of food was almost identical in the three groups; that is, 4, 4, and 4.5 gm of basal diet per day, respectively. However, to rule out completely the possibility that the low intake of basal diet was responsible for the infarcts observed, we put 6 depleted animals on 5 μg of PGA 6 times per week but restricted their basal diet intake to only 2 gm daily. These animals lost weight, the average loss being 27 gm, but all survived the maximum length of time that the negative controls had lasted, namely two weeks. The white cells rose to such a level that the animals had a normal count at the end of two weeks on the supplement.

The average gain in leukocytes was 4072 cells per mm.^{3} The animals were in good condition as regards cleanliness and activity. None showed lesions either in the spleen or anywhere else at autopsy 16 days after supplementary feeding was started. This was a very different picture, indeed, from that observed in the negative controls, which were consistently clumsy, slow, untidy, and suffered from a severe leukopenia and a high incidence of infarcts in the spleen. Both groups, however, exhibited severe alopecia. For the experimental group the average degree of alopecia at the end of the experiment was 3.

Splenic infarcts

None of the animals which received 5 μg, or more, of PGA per day showed infarcts in the spleen at the time they were sacrificed; that is, after receiving the supplement for 5 consecutive weeks. However, as 73.5% of the rats which died between the third and 4th weeks of depletion developed infarcts in the spleen, it is logical to conclude that a high percentage of the animals surviving the 4th week of the depletion period were already suffering from such lesions. This was partly confirmed by examining the spleens of 6 depleted rats that appeared very active and lively at the end of the 4th week. These animals were sacrificed and infarcts found in 4, while the spleens of the remaining 2 appeared normal. As it is possible that a high percentage of the depleted animals were suffering from infarction at the time supplementary feeding began, one cannot discard the possibility of regeneration taking place in the spleens of some of these animals during the supplementation period. Bloom and Taliaferro ('38) have reported complete regeneration of infarcts in the spleens of canaries caused by heavy infection with *P. cathemerium*. In order to confirm fully this indirect observation made by us on rats, we are now conducting experiments in which we are endeavoring to ascertain, by laparotomy and by microscopical examination of the tissues, whether regen-

eration has taken place in the spleens of depleted animals treated with PGA.

The infarcts (see fig. 2) found in the spleens of animals suffering from PGA deficiency can be classified into three general groups: (1) Total infarction, where all of the organ has been affected; (2) multiple surface infarcts, in which small concentrated areas of infarction can be seen distributed

Fig. 2 Severely infarcted spleen from a rat suffering from PGA deficiency. Two and ¾ times natural size.

through the surface of the organ; (3) terminal infarcts affecting either one end or both ends of the spleen.

Multiple surface infarcts were the most common of the three, while complete infarction was not seen very often. The character and intensity of infarction observed in the individual animals did not seem to depend on the level of PGA administered, as all three types of infarcts were observed in the negative controls as well as in the animals receiving suboptimal levels of PGA. Although PGA did not seem to in-

TABLE 2

Weights of the spleens of normal and of PGA-deficient rats

	SPLEENS WITHOUT INFARCTS			SPLEENS WITH INFARCTS		
	No. of spleens	Ave. weight of spleen	Wt. of spleen per body wt.	No. of spleens	Ave. weight of spleen	Wt. of spleen per body wt.
		gm	*gm*		*gm*	*gm*
Normal animals (8 weeks old)	65	0.5371	0.0048	..	.....	.....
Normal animals (3 to 4 weeks old)	5	0.1919	0.0046	..	.....	.....
Depleted rats receiving as supplement 5 μg PGA per day, but with the basal diet restricted to 2 gm per day (9 weeks old)	6	0.1639	0.0036	..	.....	.....
Depleted animals receiving as supplement an average of 0.5 μg PGA per day (9 to 18 weeks old)	10	0.1449	0.0018	6	0.1422	0.0025
Negative control animals (7 to 9 weeks old)	13	0.0820	0.0019	34	0.1342	0.0031

fluence the type of infarct developed, it had a decided influence on infarct prevention and also, possibly, on its cure, as has been already pointed out.

Histological examination [10] of the spleens of rats with infarcts revealed the necrotic portions of the spleen to be sharply demarcated from the more normal but intensely congested parts. In many of the sections, the splenic veins were occupied by material that probably represented a thrombus.

Splenomegaly was rarely encountered. Table 2 summarizes the data on spleen weights of normal and deficient animals.

[10] We are indebted to Dr. Enrique Koppisch, of the Department of Pathology of the School, for the histological examination of the spleens. A detailed report of the histopathology of the rat in PGA deficiency is being prepared and will be published elsewhere by Koppisch et al.

The weight of the spleen computed per unit weight of animal also appears in this table.

The total weight of the spleen, or its weight per unit weight of animal, was much higher in the case of normal animals than in the case of deficient ones. The average deficient animal suffered from atrophy of the spleen. This condition was more noticeable in the case of those negative controls which did not develop infarcts than in those whose spleens suffered from this lesion.

Induction of spleen infarction by methyl folic acid [11]

The basal diet used in this part of the present experiment had a composition similar to that of the previous ration, except that for the 2% of SST was substituted 0.85% methyl folic acid and the difference made up with sucrose. This diet was provided ad libitum to 18 rats, 21 days old. These animals made small gains in weight during a period of three weeks but then lost weight abruptly and died. They became very untidy, developed lesions in the skin, exhibited a severe alopecia, suffered from an acute leukopenia and also developed the ulcerative lesions in the central parts of the anterior surface of the tongue described by Franklin et al. ('47). In addition, 89% of these animals exhibited infarction of the spleen. Altogether the deficiency symptoms were much more dramatic than those observed in the animals which received the diet containing SST as inhibitor. The tongue lesions, however, have also been observed by us in some of the depleted rats receiving SST as inhibitor.

DISCUSSION

Taking as criteria of normalcy the rate of gain in weight, the concentration of white cells in the blood, and the condition of the spleen, it can be stated that the minimum amount of PGA required by depleted rats to regain their normal state

[11] Provided by Dr. T. H. Jukes of Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

is somewhere in the neighborhood of 5 μg per day, when fed 6 times weekly during a 5-week period. Those animals which received 20 μg per day gained weight and recovered white cells at an optimum rate, and not a single one suffered from spleen infarction.

The level of 5 μg of PGA per day, found by us to be the minimum requirement of depleted rats for normalcy, is within the range obtained recently by another investigator (Welch, '48) in the course of some exploratory experiments. He finds that the minimum amount of PGA required per day by depleted rats is somewhere between 2 and 10 μg.

As far as we can learn from the literature, the above observations regarding the development of infarcts associated with pteroylglutamic acid deficiency in rats have not been previously described. That the spleen infarction observed is a direct result of PGA deficiency seems to be well substantiated by the fact that at increased levels of PGA incidence decreased, until a dose was reached (5 μg per day) that either fully prevented or cured the lesions in a period of 5 weeks.

The possibility that the infarcts could be the result of a secondary deficiency induced by the limited intake of basal diet was ruled out by the experiments already described elsewhere in this paper. The fact that the same type of lesion was observed with two different PGA inhibitors gave further support to the theory that this abnormal condition of the spleen is one of the manifestations of PGA deficiency in the rat. It seems that a definite concentration of PGA has to be maintained in rat tissues to prevent infarct formation in the spleen.

It is well-known that the spleen is a dispensable organ. Splenectomized healthy animals live their full span of life quite as normally as those with spleens. It is not surprising, therefore, that several rats, not described in this report, fed the basal diet supplemented with a suboptimal level of PGA, lived for several months with severely infarcted spleens. It seems that the development of this lesion is not an immediate cause of death.

In view of the high percentage of animals suffering from infarction during the depletion period, it appears difficult to believe that regeneration did not take place in a substantial number of those animals having normal spleens at the end of the 5-week supplementation period. But direct proof that regeneration takes place is still forthcoming.

SUMMARY

1. Twenty-one-day-old Wistar rats, fed a PGA-deficient ration containing 2% SST as inhibitor, developed marked deficiency symptoms in an average of three to 4 weeks on the ration.

2. Full remission of the depletion symptoms was obtained by administration of 5 μg of PGA 6 times per week during a period of 5 weeks.

3. Levels of PGA below this amount induced partial remission of some, or all, the symptoms at rates that were roughly proportional to the level of PGA fed to the animals.

4. A characteristic lesion, hitherto undescribed, consisting of infarction of the spleen, was observed in a high percentage of the PGA-deficient animals. No such lesion was found in animals that had received 5 μg or more of pteroylglutamic acid per day as a dietary supplement during a period of 5 weeks subsequent to depletion.

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REPRODUCTION AND LACTATION STUDIES WITH RATS FED NATURAL AND PURIFIED RATIONS [1]

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(Received for publication April 30, 1948)

INTRODUCTION

Hayward et al. ('34) demonstrated that a corn-soybean-alfalfa ration was adequate for growing and fattening swine. Hayward and associates ('36) found that when soybeans are heated the sulfur-containing amino acids become more available to the experimental animals.

Ross et al. ('44) and Cunha et al. ('44a), using a corn-soybean oil meal-alfalfa ration, had difficulty in reproduction with swine. Few pigs were weaned and symptoms of congenital malformation were found in young pigs from second generation gilts raised on the basal ration. Often the sow died of toxemia during pregnancy. The basal ration consisted of 17.50% soybean oil meal, 76.35% yellow corn, 5.0% alfalfa meal, and 1.15% minerals.

Ross ('43) found that reproduction and lactation in rats were improved when the basal diet was supplemented (at the expense of the corn) with either fish meal, 1:20 liver concentrate, 10% additional alfalfa meal, tankage, dried brewers' yeast, soybean, lecithin or valine. Increasing the calcium and phosphorus level and adding manganese to the diet also

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the A. E. Staley Manufacturing Company, Decatur, Illinois.

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gave slightly better results. Regularity in estrus cycle was obtained when B_1, B_6, niacin, α-tocopherol and shark liver oil were added to the basal ration.

Cunha ('44) found differences in the growth of rats which he attributed to variations in quality of dietary protein. The reproduction and lactation factor (or factors) was shown to be stored or retained in the rat for the production of three successive litters. The basal ration supplemented with 5% acid-washed casein plus choline gave normal reproduction and lactation, but 5% vitamin-free commercial casein [2] did not. Supplements to the basal ration of kidney residue plus inositol, or inositol plus choline plus B_6 plus 2% 1:20 liver powder, or 2% solubilized liver extract plus inositol, stimulated reproduction and lactation. The addition of B_6, choline, inositol, biotin, calcium pantothenate and folic acid was found essential for normal growth in rats.

In further studies Spitzer et al. ('46a) showed that this basal ration was inadequate for reproduction and lactation in rats; in this study toxemia symptoms similar to those occurring in swine fed this diet were noted. The inadequacy of the ration produced lactation failure and some reproduction difficulties.

Spitzer ('47) formulated the basal "T" ration, composed of 20% soybean oil meal, 65.84% yellow corn (ground), 5.00% dehydrated alfalfa meal, 2.16% salts (Spitzer and Phillips, '46a), 2.00% Salts IV (Phillips and Hart, '35), and 5.00% corn oil, plus the following per 100 gm of ration: 0.4 gm L(+)—cystine, 0.04 gm α-tocopherol, 0.2 gm halibut liver oil, 0.3 gm inositol, 0.3 gm choline chloride, 5 mg menadione,[3] 20 mg ascorbic acid, 30 mg p-aminobenzoic acid, 0.1 mg folic acid, 0.01 mg biotin, 1 mg B_1, 1 mg B_2, 1 mg B_6, 5 mg calcium pantothenate and 5 mg niacin. This fortified basal diet improved the reproduction and lactation performances of female rats by enabling them to wean 32% of their young. The further addition of 5% soybean oil meal at the expense of corn was

[2] Labco.

[3] 2-methyl-1,4-naphthoquinone.

detrimental and resulted in the weaning of only 17% of the young. Bethke et al. ('46) found that supplementation of an all-plant basal diet for laying hens with soybean oil meal resulted in poor hatchability. Whitson et al. ('46) observed a decrease in the hatchability of eggs as the soybean oil meal was increased from 0 to 40% in the diet, despite the apparent adequacy of the dietary factors affecting hatchability. Spitzer ('47) demonstrated that reproduction and lactation were further improved by supplementing the basal "T" ration with either 5% crude casein, 5% fish meal, 0.3% soybean lecithin, 5% 1:20 liver powder, or 10% additional alfalfa meal.

This paper reports further studies on reproduction and lactation in rats fed our basal corn-soybean oil meal-alfalfa diet.

EXPERIMENTAL

Procedure

In this work Sprague-Dawley albino rats and their weanlings weighing over 30 gm at the time of weaning were used. The females were conditioned or standardized carefully as outlined below. The rats were raised to maturity on the stock ration. Prior to mating all the female rats to be used were depleted for three weeks on the basal ration. They were then mated for the first time, at 110–120 days of age. The rats were permitted to bear three litters and were then discarded. Only healthy appearing rats were used for the experiments. The female rats were given a three-week rest period between weaning their young and remating; during this period the animals received the stock ration. A record was kept of the age, the number of litters produced and the strain source (Sprague-Dawley or inbred rats of Sprague-Dawley origin). In each experiment, with the exceptions noted, only rats similar on all three counts were used.

The cages used for mating were large, wire-mesh, false bottom, galvanized steel cages. A maximum of 5 females per cage was allowed during mating. The males used for mating were changed each week. When the females became pregnant

(detected by marked weight gain), they were isolated in smaller galvanized steel cages with galvanized steel bottoms. Clean wood shavings of soft texture and free from sawdust were used for nesting. The wood shavings were changed once a week.

Since early lactation failure occurred in the females fed the basal ration, lactation performance and the weaning of young are best portrayed by the per cent of young weaned. This figure was obtained by dividing the number of young weaned by the number of young born. In order to reduce as much as possible the effect of litter size upon the growth rate of the young, all litters were reduced 48 hours after birth to six young. Despite this precaution, in most cases the litter load was not entirely equilibrated. The young were weaned at 21 days of age. The diets were closely similar in caloric value. Since the object of this study was qualitative and not quantitative in nature, and in view of the inherent difficulties of controlling food intake and food waste by young rats, food consumption data were inaccurate and therefore all rations were fed ad libitum. Birth weights were not recorded, since the size of the litter overshadows dietary influence upon the weight of young at birth.

In the preliminary experiments (Maruyama, '48) to test the stimulatory effect of added casein or casein hydrolysates, only slight, if any, stimulatory effect upon reproduction and lactation was obtained. Supplementing basal ration "T" with either crude or purified casein did result in less loss of weight by the suckling mother and somewhat greater gains in body weight of the nursling young. Hydrolysis of the casein by either acid or enzymes apparently destroyed the growth-promoting principle. The addition to the basal "T" ration of the 19 amino acids equivalent to 5% casein resulted in excellent reproduction and lactation performances in preliminary tests with rats; however, the growth factor for the young was not present in adequate amounts, if at all. Thus the preliminary results suggested that the missing lactation

factor in the corn-soybean oil meal-5% alfalfa diet may actually have been low levels of certain of the amino acids.

Experiment I

With these indications from preliminary experiments in mind, the amino acid content of our natural ration was calculated on the basis of figures for the 10 essential amino acids of the foods used here given by Block ('45) and by Block and Mitchell ('46–'47). It was found that lysine and methionine were lower in the basal "T" ration than in the basal "T" plus 5% crude casein. Furthermore, if the requirement of the rat for the individual amino acids was estimated on the basis of the work of Griffith and Farris ('42), then both lysine and methionine were lower in the basal "T" ration than the recommended dietary intake. When basal "T" was supplemented with 5% casein, the amounts of the two critical amino acids were increased to approximately the recommended levels. The effects of supplementing the basal ration with methionine and lysine equivalent to 5% casein, as well as with folic acid, pancreatin, and corn steep liquor additions, were studied. Pancreatin was used in order to minimize the effect of the antitryptic factor if present in the soybean oil meal; it has been reported by Ham et al. ('45) to be in raw soybean meal.

The results are given in table 1, experiment I. The addition to the basal "T" ration of 0.5% L(+)-lysine, 0.3% DL-methionine, or 0.5% L(+)-lysine plus 0.3% DL-methionine gave increased performance, in the order mentioned, in our inbred rats, with normal reproduction and lactation in the case of the last-mentioned supplement. The addition of pancreatin did not have a beneficial effect. The omission of folic acid from the basal "T" ration produced a marked inhibitory effect on reproduction and lactation, and in two of the females resorption occurred. The addition of 5% corn steep liquor had no beneficial effect on lactation as indicated by the per cent of young weaned; however, the growth rate of the young may have been stimulated by this ingredient.

TABLE 1

The effects upon reproduction of various supplements to the corn-soybean meal-alfalfa hay ration

	RATION	NO. OF FEMALES	RATS PREGNANT	NO. OF LITTERS	TOTAL NO. OF YOUNG BORN	RATS WEANED [1]	AVERAGE WEANING WEIGHT [2]	AVERAGE WEIGHT CHANGE OF MOTHER [2]	SIGNIFICANCE [3]
			%			%	*gm*	*gm*	
				Experiment I					
1.	Basal "T"	10	100	9	72	46	36.4	+ 27.5	....
2.	Basal "T" + 0.5% L(+)-lysine	10	90	9	65	58	37.4	+ 8.9	2.72
3.	Basal "T" + 0.3% DL-methionine	8	100	8	58	64	38.5	+ 4.5	5.21 [4]
4.	Basal "T" + 0.5% L(+)-lysine + 0.3% DL-methionine	9	100	9	64	72	35.8	+ 6.4	10.58 [5]
5.	Basal "T" + 1% pancreatin	9	100	9	52	48	37.0	+ 11.0	0.185
6.	Basal "T" minus folic acid	10	100	8 [6]	41	34	36.0	— 4.0	1.03
7.	Basal "T" + 5% corn steep liquor	8	87.5	7	48	42	40.2	+ 22.6	0.04
				Experiment II					
1.	Basal "T"	8	87.5	7	56	29	31.7	— 22.7	....
2.	Basal "T" minus folic acid	8	100	7 [6]	48	0	...	...	1.42
3.	Purified ration (with folic acid)	4	100	4	27	59	42.8	+ 32.5	....
4.	Purified ration (minus folic acid)	5	100	3 [6]	19	10	38.0	+ 29.0	9.20 [5]

[1] Per cent weaned calculated on basis of the number of young born.

[2] The growth and weight change data of the mother rats are of doubtful significance because of the unequal "litterload."

[3] Chi square significance data were calculated from the "per cent weaned" column and the values shown are compared with the performance record of group 1, basal "T."

[4] P value exceeds 5% level.

[5] P value exceeds 1% level.

[6] Resorption occurred in the remainder of the females.

If the per cent weaned was calculated on the basis of the number of live young at 48 hours it appeared that the addition of amino acids improved lactation, since 73% of the young alive at that time were weaned from mothers fed the basal "T" ration as against 83%, 86%, and 96% weaned from mothers fed the basal "T" ration supplemented with lysine, methionine or lysine-methionine, respectively.

Experiment II

The study of the effect of folic acid on reproduction and lactation was repeated in a second experiment. Many workers believe that folic acid is synthesized in limited amounts by the intestinal flora of the rat. They believe also, that when the carbohydrate of the purified ration is sucrose, the synthesis of folic acid as well as other "B complex" vitamins is reduced to a minimum. Therefore, a purified ration developed by Sporn et al. ('47) was used, with a slight modification for this experiment. It consisted of sucrose 73%, casein [4] 18%, corn oil 5% and Salts IV (Phillips and Hart, '35) 4%, plus the following vitamins in milligrams per 100 gm of ration: B_1, 0.3; B_2, 0.3; B_6, 0.2; niacin, 2; calcium pantothenate, 2; folic acid, 0.025; biotin, 0.01; inositol, 0.01; choline chloride, 100; and *p*-aminobenzoic acid, 25. Instead of feeding 10 mg of α-tocopherol per rat at the beginning of the experiment and two drops of one to 4 dilution of oleum percomorphum per week, the vitamins E, A and D were incorporated into the ration and folic acid was increased from 25 µg to 100 µg per 100 gm of the ration. Alcohol-extracted casein was used in place of the commercial preparation.[5]

The results are given in table 1, experiment II. The Sprague-Dawley rats on basal "T" weaned 29% of their young, while those on basal "T" minus the folic acid weaned 0%. One resorption occurred. Thus the essentiality of folic acid was again demonstrated. When the purified ration with

[4] SMACO.

[5] See footnote 4.

[3] P value exceeds 5% level.
[4] P value exceeds 1% level.
[5] Resorption occurred in the remainder of the females.

folic acid was used, 59% of the young were weaned, and only 10% when the folic acid was omitted from the ration. Resorption occurred in two out of the 5 rats used.

It seems evident from these data that folic acid is a factor essential for normal reproduction and lactation in the rat when either a purified or natural ration is employed.

DISCUSSION

Spitzer ('47) believed an unknown factor or factors necessary for reproduction and lactation in the rat to be present in casein. Johnson et al. ('42) have shown the presence in casein of a chick growth factor. Woolley and Collyer ('46) demonstrated that strepogenin is essential for optimal growth in mice. Scott et al. ('47) showed that the strepogenin found in 2.2% crude casein or in 10% yeast produced a two-fold gain in chick growth when the source of protein in the purified diet was 20% egg albumin (autoclaved) and 10% gelatin.

Stimulation of reproduction and lactation equivalent to that obtained by the addition of 5% crude casein to the basal "T" ration formulated by Spitzer ('47) was obtained when the 19 amino acids equivalent to 5% casein were substituted for the casein. Addition of 0.5% L(+)-lysine and 0.3% DL-methionine to the basal "T" ration brought about excellent reproduction and lactation in the rat; thus it appears that such supplementation provides additional stimulation for these functions. The requirement of extra methionine for the adequacy of soybean oil meal protein has been demonstrated by Almquist et al. ('42). Russell et al. ('46) showed that methionine enhanced growth in rats fed soybean oil meal rations.

In our studies folic acid, among other factors, has been shown to play an important role in the reproduction and lactation of the rat. Possibly the requirement for folic acid increased during the gestation period and the amount synthesized in the digestive tract plus the small amount in the constituents of the diet were not sufficient to promote normal gestation and parturition. Hence, resorption occurred fre-

quently. Recently, Lillie and Briggs ('47) determined by chick assay the folic acid activity in common feedstuffs and reported alfalfa meal to contain 11.3 μg per gram and liver and yeast 50 μg per gram. This may explain in part the stimulation obtained by supplementing alfalfa meal, liver or yeast to the basal diet by Ross ('43), Cunha ('44), and Spitzer ('47). Cerecedo et al. ('44, '47) showed the beneficial effect of folic acid on lactation in mice, using a highly purified ration. Fenton and Cowgill ('47) found that some stimulation for reproduction and lactation in their highly inbred strain of mice resulted when folic acid was added to a purified diet. Bowland et al. ('48) reported stimulation from folic acid for reproduction and lactation in rats but not for growth.

Due to the unequal number of young that the mother rats nursed to weaning, the data on the average weaning weight of the young and the weight changes in the mother rats during the gestation and lactation period can be considered only as suggestive but not significant in determining the adequacy of the diet in these experiments, a point stressed by Nelson and Evans ('47).

Efforts to supplement adequately a yellow corn-soybean oil meal-5% alfalfa ration in order to stimulate reproduction and lactation in the rat have shown that choline, B_6, inositol (Ross, '43; Cunha, '44) and biotin (Spitzer, '46b) improve the ration as regards these important functions. Further improvement has now been attained by additional supplements of 0.5% L (+)-lysine and 0.3% DL-methionine and folic acid.

SUMMARY

1. Standardization of the experimental animal was found to be essential for reproduction and lactation studies.

2. Supplementing the basal "T" ration with 0.5% L(+)-lysine and 0.3% DL-methionine led to excellent reproduction and lactation in rats. The weight of the young weaned on this ration was less than that of young weaned on the casein-supplemented ration.

3. Supplemental folic acid appeared to be essential for normal reproduction and lactation in our female rats fed the corn-soybean-5% alfalfa ration or a purified ration.

4. The addition of 1% pancreatin or 5% corn steep liquor to the ration did not have any stimulative effect on reproduction or lactation, but 5% corn steep liquor did stimulate growth in the suckling young.

5. From these studies it is evident that in addition to other factors demonstrated to be helpful in reproduction and lactation — i.e., choline, B_6, inositol, and biotin — the amino acids L(+)-lysine and DL-methionine, and folic acid, are essential for good reproduction and lactation in female rats fed basal ration "T," which is essentially a corn-soybean oil meal-5% alfalfa ration.

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THE LACTATION RESPONSE AS LIMITED BY FEEDS PRODUCED UNDER TWO SYSTEMS OF SOIL FERTILIZATION

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ONE FIGURE

(Received for publication April 29, 1948)

Observations of certain workers indicate that varying levels of soil fertility have affected through the medium of plants the nutrition and milk production of dairy cattle (Pipers, '09; Green, '25). In other studies the weight gains of animals have been influenced (Theiler et al., '20, '24; Crampton, '34; Albrecht and Smith, '41; Webb et al., '46; Turner et al., '46). Improved palatability (Nevens, '41; Archibald et al., '43) and an increased apparent digestibility of certain forages (Nehring, '38; Eheart and Pratt, '42) have been attributed to soil fertilization; in other work the biological value of proteins in barley (Nehring and Schramm, '40) was not improved by such means. Other investigators report improved animal reproductivity, health and longevity resulting from soil fertilization (Hunt, '27; Tallarico, '31; Scheunert et al., '34).

The effects of varied levels of soil fertility upon animals have been investigated largely through the feeding of grasses or through range grazing studies. Because of the relatively meager evidence relating to such levels as they affect cereal grains and legumes, and to their ultimate influence upon the

[1] The data of this paper were taken from a thesis submitted by this author to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

nutrition of animals, further study of the general problem seemed justified.

The object of this investigation was to study the lactation response in the rabbit as it is influenced by the nutrient content of feeds produced on Cisne silt loam fertilized with phosphorus and potassium and with or without calcium.

The cooperation of the Department of Agronomy of the University of Illinois made this study possible by supplying the cereal grain and legume roughages fed, which were grown on the Soil Experiment Field located at Newton, Illinois. This field was established in 1912. Since the time of its establishment accurate records have been kept of the fertilizers applied and their effects upon the productivity of the soil as shown by crop yields (Bauer et al., '45).

EXPERIMENTAL

The effects of two series of fertilizer applications upon the nutritive value of wheat grain, lespedeza hay, and soybean hay were studied through the use of paired feeding trials with lactating rabbits and their nursing young. The fertilizer series and the amounts of each fertilizer applied per acre since the field experiments were started were as follows: Series 1, raw rock phosphate, 4 tons; kainit, 2.25 tons plus 1,000 pounds of muriate of potash. Series 2 was similar to series 1 in that 4 tons of raw rock phosphate, 2.25 tons of kainit, and 1,000 pounds of muriate of potash were used, but differed from series 1 in that 12 tons of limestone were also applied. For the sake of clarity and brevity, series 1 will be referred to as the PK and series 2 as the PK + Ca treatment.

Trial 1: Lespedeza hay and wheat studies

Equal parts of ground wheat grain and ground Korean lespedeza hay (1945 crops), produced either on the PK or the PK + Ca series, and 1% sodium chloride (C.P.) were used in making up two rations. The first was known as the PK ration, the second as the PK + Ca ration. The lespedeza

produced under each soil treatment was cut on the same day, while the crop was in the early to medium bloom stage. The hay was cured under good hay-making conditions and stored with a minimum of leaf loss. Before grinding the lespedeza all of the foreign matter, chiefly weeds, was removed. The lespedeza contents of the forage as harvested from the PK and PK + Ca-treated plots were 33.3 and 71.5%, respectively.

The total nitrogen content of the lespedeza hay from the PK-treated plots was 1.96% and for the hay from the PK + Ca soil treatment, 2.36%. The two lots of wheat contained 1.53% and 1.94% nitrogen, respectively, for the PK and the PK + Ca treatments.

The percentages of dry matter, total nitrogen, and ash for the two rations as fed were (in the order named) 89.3, 1.74 and 4.6 in the PK ration and 89.9, 2.16 and 4.4 in the PK + Ca ration.

The rabbit was used as the experimental animal. Previous investigations (Crampton et al., '40; Smith and Albrecht, '42) with the rabbit have shown it to be similar to cattle or sheep in its ability to utilize feeds.

Six pairs of litter-mate New Zealand white females and two related males were used. The female of each pair was identified by a number followed by the letter "—O" if fed the PK ration, or "—W" if fed the PK + Ca ration.

Throughout the study, the paired feeding technique was used to equalize food intakes (Mitchell and Beadles, '30). After a three-week preliminary feeding period in which the experimental rations were fed, the animals were mated. Each male was mated to two females daily with only one service per female being permitted. This use of the males was not believed to be excessive (Jones and Hays, '18).

Though the females were mated at essentially the same time, because of the variability in the length of the gestation periods age in the young was calculated in days from the probable time of conception. In most instances the young were born by the end of the 31st day after conception. As soon as possible after birth the young were counted and

weighed. Usually, by the third day the litters were standardized to 6 young or less.

The feeders used were mounted high enough to prevent the young from gaining access to the rations fed. Thus the milk of their mothers was the only source of nutrients for the young.

Daily weights of litters were taken until the 46th day from the probable date of conception, at which time the litters were sacrificed. The animals of a litter were weighed individually and were measured for body length. After removal of gastrointestinal contents the carcasses were weighed, frozen and later ground for analysis.

The individual carcasses were analyzed for dry matter, total nitrogen and ash. In all, 46 carcasses were analyzed, comprising an equal number from each of the two groups compared. Because the entire litter of doe 8-O (PK) died on the 35th day after conception, the observations taken for doe 8-W (PK + Ca) and her litter have been omitted from the reported data.

Dry matter was determined by holding 1–2 gm samples of ground carcass at 100°C. for 4 hours. Total nitrogen was determined by the official Kjeldahl method (A.O.A.C., '40). Samples for ashing were placed in a muffle furnace. The temperature was slowly raised to 575–600°C. and held for 6 hours.

Trials 2 and 3: Soybean hay and wheat studies

Trials 2 and 3 differed from trial 1 in that excellent quality soybean hay was used to replace the lespedeza hay in the rations fed. The soybeans were grown under the same conditions as was the lespedeza used in the first trial.

The total nitrogen in the wheat (1946 crop) from the PK-treated soil was 1.79%, compared with 2.01% for that produced on the PK + Ca-treated plots. The soybean hay from the PK-treated soil contained 2.39% total nitrogen in contrast to 3.02% for hay from the PK + Ca-treated soil.

The ration used in trials 2 and 3 contained the same proportions of ground wheat, ground soybean hay, and sodium

chloride as were used in trial 1. The percentages of dry matter and total nitrogen in the rations as fed were 88.7 and 2.06 respectively, for ration PK, and 89.1 and 2.49 respectively for the PK + Ca ration.

TABLE 1

Weights of does and their food intakes

DOE NUMBER	DOE WEIGHTS AT TIME OF:			FEED CONSUMED DURING:	
	Mating	Parturition	Litter sacrifice	Gestation	Lactation
	kg	*kg*	*kg*	*kg*	*kg*
	Trial 1: Wheat and lespedeza hay study				
1-O [1]	3.9	3.8	3.5	3.1	2.0
1-W [2]	3.9	3.8	3.6	3.2	2.0
2-O [1]	4.0	3.9	3.7	3.7	2.2
2-W [2]	4.3	3.9	3.8	3.6	2.3
3-O [1]	4.3	4.1	3.9	3.4	1.8
3-W [2]	3.7	3.5	3.5	3.4	1.8
6-O [1]	3.9	4.0	3.7	4.1	2.6
6-W [2]	4.3	4.3	3.9	4.1	2.6
7-O [1]	3.9	3.9	3.4	3.6	2.6
7-W [2]	3.6	3.8	3.4	3.6	2.6
	Trial 2: Wheat and soybean hay study				
1O2 [2]	4.9	4.5	4.5	2.3	2.6
1W2 [1]	4.4	4.1	4.0	2.3	2.7
5-2 [2]	3.9	3.9	4.2	4.8	2.8 [3]
8W2 [1]	3.4	3.6	3.7	3.2	2.8 [3]
	Trial 3: Wheat and soybean hay study				
5-3 [2]	3.7	3.7	3.8	3.2	2.7 [3]
2W3 [1]	3.7	3.5	3.7	4.2	2.7 [3]
Average [1]	3.9	3.8	3.7	3.4	2.4
Average [2]	4.0	3.9	3.8	3.5	2.4

[1] Ration from PK-treated soil.
[2] Ration from PK + Ca-treated soil.
[3] Paired only during lactation.

Approximately 6 months after the completion of the wheat and lespedeza feeding studies the same females were again placed on experiment. In these studies the females were reversed with reference to the soil treatments as affecting the rations.

RESULTS

The PK + Ca ration that contained lespedeza appeared to be slightly more palatable than the PK ration. This was indicated by the quantity of feed that remained in the feeders

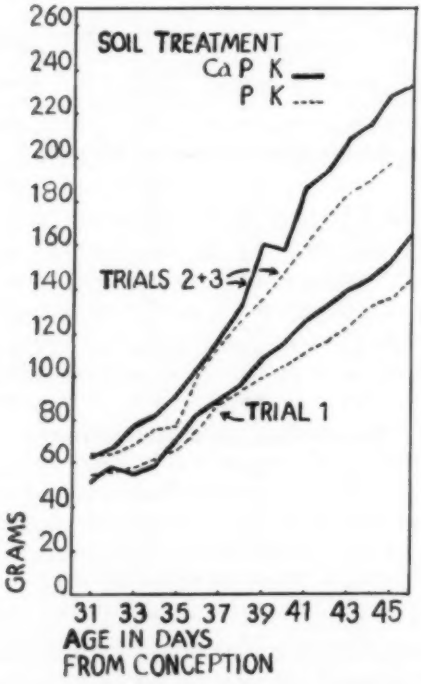

Fig. 1 The average daily weights of nursing rabbits whose mothers, in trial 1, were fed lespedeza hay and wheat and in trials 2 and 3, soybean hay and wheat. All feeds were grown on a soil that had been treated with two different combinations of fertilizers.

each day. Four of the 5 does receiving ration PK, which contained lespedeza, limited the feed intakes of their pair mates. When the soybean hay and wheat rations were fed, the tendency to limit the food intake of pair mates was about evenly divided between the two soil treatments.

A study of the average weights of the lactating females as shown in table 1 indicates that the average total weight losses while on the two rations were approximately equal. The average losses were 0.24 and 0.21 kg respectively for the PK and the PK + Ca treatments. These losses occurred from the time the females were mated until the time the young were sacrificed, 46 days later.

TABLE 2

Average body measurements and carcass composition of litters in trial 1

LITTER NUMBER	NUMBER OF YOUNG	BODY MEASUREMENTS			CARCASS COMPOSITION		
		Length	Full wt.	Empty wt.	Dry matter	Total nitrogen	Ash
		cm	*gm*	*gm*	*gm*	*gm*	*gm*
1-W [1]	6	18.6	135	124	29.0	3.3	4.2
1-O [2]	6	17.2	110	102	25.2	2.8	3.6
2-W	3	20.0	193	175	40.9	4.9	6.3
2-O	2 [3]	18.3	163	141	33.2	3.9	5.0
3-W	4	19.9	177	156	35.2	4.0	5.0
3-O	4	18.3	143	126	28.4	3.4	4.0
6-W	5	21.3	219	198	54.7	5.3	5.9
6-O	5	19.9	189	173	49.7	4.5	5.8
7-W	5 [4]	20.1	170	154	40.2	4.4	5.6
7-O	6	19.0	153	146	39.1	4.2	5.2
-W	23	19.9	176	159	39.6	4.3	5.4
-O	23	18.5	149	136	35.3	3.7	4.7

[1] "-W" = PK + Ca ration (wheat and lespedeza).
[2] "-O" = PK ration (wheat and lespedeza).
[3] One animal died 4 days before the litter was sacrificed.
[4] One animal died two days before the litter was sacrificed.

The pairs of mating totaled 17. Of the 17 females that received the PK rations, 14 bore litters averaging 6.43 young per litter, with three of the females failing to conceive. Twelve of the 17 females receiving rations PK + Ca produced an average of 7.75 young per litter, with the 5 remaining females failing to have litters. Birth weights of the individual animals in any litter were inversely related to the number of animals in the litter.

The average daily weights of the litters of nursing dams receiving the PK + Ca rations were larger than those of the litters of their paired litter mates. The differences in the average daily weights of litters were apparent after the 38th day from conception, as is shown in figure 1.

As is indicated in table 2, the average body length for the 23 young in trial 1, whose dam had been fed ration PK, was 18.5 cm as compared with an average body length of 19.9 cm

TABLE 3

Average body measurements of litters in trials 2 and 3

RATION	LITTER NUMBER	NUMBER OF ANIMALS	BODY MEASUREMENTS:		
			Length	Full wt.	Empty wt.
			cm	*gm*	*gm*
		Trial 2 [1]			
PK	1W2	3	20.8	230	209
PK + Ca	1O2	3	21.4	251	218
PK	8W2	4 [2]	20.2	219	186
PK + Ca	5-2	5	21.1	210	181
		Trial 3 [1]			
PK	2W3	3 [3]	19.2	205	182
PK + Ca	5-3	3	23.1	289	257
PK		10	20.0	218	191
PK + Ca		11	21.7	242	211

[1] Wheat and soybean hay studies.

[2] One animal died two days before the litter was sacrificed.

[3] One animal in this litter was quite small, weighing only 92 gm at the time of sacrifice.

for those whose dams had been fed ration PK + Ca. Although a smaller number of young survived in trials 2 and 3, their body lengths for the PK and PK + Ca rations were 20.0 and 21.7 cm respectively.

The average weight of stomach and intestinal contents of the 23 PK young in trial 1 was 12.6 gm, as compared to 16.8 gm for the 23 young reared from does on PK + Ca rations. The same trend appears in trials 2 and 3 where the gastro-

intestinal contents were 27 and 31 gm, respectively, for the PK and PK + Ca treatments.

The average quantities of dry matter, total nitrogen, and ash deposited in the carcasses, as shown in table 2, were 35.36, 3.86 and 4.79 gm respectively for those animals affected by the PK treatment. The percentages of these same components in the carcasses were: dry matter, 25.52; total nitrogen, 2.78; and ash, 3.51. The quantities of these deposition products were in direct contrast to those for the PK + Ca animals. In this group the deposited dry matter, total nitrogen and ash averaged 39.66, 4.42 and 5.36 gm, respectively. Percentage values for these same components in the order named were 24.65, 2.75 and 3.39.

TABLE 4

Analysis of the variance between the PK and PK + Ca treatments as affecting carcass measurements and composition of animals in trial 1

CARCASS FACTORS ANALYZED	F	COEFFICIENT OF VARIATION
		%
Body length	30.3 [1]	4.5
Gastrointestinal contents	12.9 [1]	26.6
Body weight (empty)	19.4 [1]	11.6
Dry matter deposited	8.0 [1]	13.6
Nitrogen deposited	26.5 [1]	19.0
Ash deposited	10.5 [1]	11.9
Dry matter (%)[2]	13.9 [1]	3.2
Nitrogen (%)	.0025	7.9
Ash (%)	2.9	6.6

[1] Significant at the 1% level of probability.
[2] Significantly greater in the young of the PK-nourished mothers.

An analysis of the variance in the carcass observations in the first trial is summarized in table 4. The variance between treatments with respect to body lengths, gastrointestinal contents, empty carcass weights, quantity of dry matter, total nitrogen, and ash deposited was in every instance sufficiently greater than the error variance to be highly significant at the 1% level of probability. The variance between treatments

with respect to the percentage of total nitrogen and ash was only slightly greater than the error variance and was therefore not significant.

Although the differences between the values for average body lengths, gastrointestinal contents and empty carcass weights of the young produced in trials 2 and 3 showed the same trends as did those in trial 1, the number of degrees of freedom was not sufficiently large to establish the significance of the differences observed.

DISCUSSION

The causes of the wide differences in the nutritive values of the feeds grown under the two systems of soil treatment are not apparent from the data presented in this paper. It is possible that a simple correction of the protein disparity might suffice to equalize the two rations. On the other hand, it seems probable that the differences are more deep-seated than a mere difference in the nitrogen contents of the rations. Did the two systems of soil treatment cause differences in the physical as well as the chemical structure of the plant tissues? Did the addition of calcium to one series of plots enable the plants grown thereon to absorb more of some of the trace minerals? In order to determine the underlying causes of the differences which may be produced in animals by the two systems of soil fertilization, further careful research is needed. Such research is now in progress.

SUMMARY

Differences in the nutritive values of lespedeza hay, soybean hay, and wheat grain were carefully appraised through the lactation responses of rabbits. The use of doe litter mates and the paired feeding technique made such an evaluation possible.

Data are reported for 17 pairs of pair-fed, mated, doe rabbits whose rations were made up of feeds produced on a single soil type; and for 8 pairs of litters produced by these does, to an age of 46 days from conception. One ration was made

up of feeds grown on PK-fertilized soil; the other consisted of feeds grown on a similar soil fertilized with PK + Ca.

The PK + Ca rations were found to be superior to the PK rations in the following respects:

1. In trial 1 they appeared to be more palatable. No noticeable differences in palatability were observed in trials 2 and 3 when soybean hay replaced lespedeza hay.
2. The average litter size showed some tendency to be larger.
3. Average daily weights of the young were greater after reaching an age of 38 days from conception.
4. An analysis of the variance of the trial 1 data showed that body length, gastrointestinal contents, and the quantity of dry matter, total nitrogen, and ash deposited in the young fed solely on the milk of mothers fed the PK + Ca ration were significantly greater than in their PK-nourished pair mates.

Differences in the percentages of carcass nitrogen and ash were not significant.

ACKNOWLEDGMENTS

The author's sincere appreciation is expressed to Prof. W. L. Burlison for making certain facilities of the Department of Agronomy available for this investigation; to Prof. P. E. Johnson for his supervision of the harvest and storage of the crops from the plots; to Prof. F. C. Bauer for information regarding the history of the Soil Experiment Field; to Prof. E. Roberts for his generous loan of feeding equipment; to Profs. T. S. Hamilton and W. W. Yapp for helpful suggestions; to Profs. L. E. Boley and H. Hardenbrook, Jr. for their valuable assistance in maintaining the general health of the animals; and to Prof. G. W. Salisbury for his assistance in the final preparation of the manuscript.

Mr. G. C. Edwards gave helpful suggestions regarding rabbit care. The authors are very grateful to Mr. N. F. Mosser, Livestock Foreman, and Mr. Fay Campbell, Farm Foreman, for assistance in the preparation of the rations and the care of the animals.

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THE EFFECT OF PREVIOUS DIET ON THE ABILITY OF ANIMALS TO DO WORK DURING SUBSEQUENT FASTING[1]

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ONE FIGURE

(Received for publication June 21, 1948)

The present investigation was begun in order to determine the effect of diet on the ability of animals to withstand adverse conditions during subsequent fasting. Previous work has indicated that the metabolism of the peripheral tissues of animals is affected by the prevailing major energy component of the diet. This effect tends to persist during subsequent fasting (Guest, '41; MacKay, Carne, Wick and Visscher, '41; Samuels, Reinecke and Ball, '42; Roberts, Samuels and Reinecke, '43). Experiments were therefore set up in which the daily caloric intake of all groups of animals was maintained at the same level by forced feeding but was supplied largely by either carbohydrate, fat, or protein. In all groups sufficient protein was fed to maintain an adequate amino acid supply. The animals were placed in metabolism cages which also recorded activity. Voluntary activity and

[1] The research reported in this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained herein are those of the authors; they are not to be construed as necessarily reflecting the views or endorsement of the War Department.

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nitrogen balances were determined during the feeding period. Fasting was then begun, and at intervals the amount of exercise required to produce complete exhaustion was determined. The animals were fasted until they died of either exhaustion or starvation.

METHODS

In the present experiments, male rats obtained from Sprague-Dawley, Inc., were placed in combined activity and metabolism cages when they weighed 150–175 gm. The cages were designed by Dr. Reinecke. Each consists of a cylindrical revolving cage of low inertia which drives a counting mechanism that adds every inch of movement in either direction around the axis. The cage in turn can be driven by sprocket and chain from a variable speed motor. To prevent the animal from riding the cage, a baffle is introduced at the top. Below the cage are a funnel and a collecting mechanism for recovering urine and feces separately. All of the parts which come in contact with the animal or its urine and feces are built of stainless steel, thus reducing the possibility of contamination with metals and the consequent catalysis of the destruction of certain organic molecules.

After being placed in the cages, the rats were fed a stock diet of calf meal ad libitum until they weighed 250 gm or more. During this time records of voluntary activity were kept. The rats were then placed on one of the diets shown in table 1. These were fed by stomach tube in equicaloric amounts, the usual intake being 52 calories per day, which was divided into two or three feedings. Forced feeding eliminated the variability in rate of food intake which has been shown by Tepperman, Brobeck and Long ('43) to have an influence on the type of metabolism. Records of voluntary activity were also kept during the periods on the experimental diets.

At the end of 4 weeks on the experimental diets the animals were fasted. Exhaustion tests were run from one to 4 days later and thereafter at intervals of one week until the animals

died. Voluntary activity was recorded during the intervals between exhaustion tests.

In two experiments blood sugar samples were taken during the feeding period. In all experiments blood sugar levels were determined 12 hours after beginning the fast and before and after each exercise period; sometimes at more frequent

TABLE 1

Diets used in stomach tube feeding

	HIGH FAT	HIGH CARBOHYDRATE	HIGH PROTEIN
Lactalbumin, Labco (gm)	30	30	263.25
Gelatin, U.S.P. (gm)	30	30	30
Corn oil (gm)	105.8	3	3
Dextrin (gm)	0	233.25	0
Salt mixture, U.S.P., no. 1 (gm)	12.0	12.0	12.0
Oleum percomorphum, reinforced [1] (drops)	12.0	12.0	12.0
Vitamin concentrate [2] (gm)	6.0	6.0	6.0
Water to make (ml)	900	900	900
Calories per ml	1.30	1.31	1.30
Caloric distribution: (% of total calories)			
Protein	19.5	19.5	92.4
Fat	80	2.7	2.7
Carbohydrate	0.5	77.8	4.9

[1] Mead Johnson and Company.

[2] Lederle's Lederplex. According to label, 6 gm contained:

Thiamine hydrochloride	24 mg	Calcium pantothenate	36 mg
Riboflavin	24 mg	Choline	240 mg
Nicotinamide	120 mg	Inositol	120 mg
Pyridoxine	2.4 mg	Folic acid	2.4 mg

Plus other water-soluble extractives from 96 gm liver.

intervals. The sugar was determined by the Reinecke modification of the Folin-Malmros method (Reinecke, '42).

Urine and feces were collected twice a week during the feeding period. A small amount of sulfuric acid and a layer of toluene were used as preservatives. During fasting the urine and feces were saved, up to the time of forced exercise. They

were then collected separately for the period of exercise and the succeeding 12 hours. This procedure of separating the samples during voluntary exercise from those of the periods of forced exercise was continued until the death of the rat. The feces and urine were each analyzed for nitrogen by the Kjeldahl method.

RESULTS

In all tables mean values are given for each group. In most instances the standard error of the mean is also given. Student's "t" test as applied to small groups has been used to determine the probability of the significance of differences observed.

Activity during the feeding period

Table 2 gives the average voluntary activities of the experimental animals, both during the feeding periods and during fasting.

The outstanding point shown is the change which occurred when a diet high in any one food factor was substituted for a mixed diet. The greater activity did not seem to be due to some special ingredient of the stock calf meal mixture, since the rate was maintained when, in experiment 2, the rats were transferred to a force-fed diet consisting of a mixture of equal parts of the high carbohydrate and high fat diets. Later, when these rats were transferred to either the high carbohydrate, high fat or high protein diet, the voluntary activity dropped to approximately one-half the rate on the force-fed mixed diet, a drop similar to that seen when the rats were transferred directly to the specific diets from the calf meal. This change was highly significant by Student's "t" test. On the other hand, there was no difference of any magnitude between the three diets high in one factor as a source of energy. Apparently a rat desires physical activity most when it is utilizing all three foodstuffs.

TABLE 2

Voluntary activity of rats during various types of feeding and periods of fasting

DIET	RATS FED	REVOLUTIONS PER DAY								
		Calf meal ad libitum	Force-fed mixed diet	Force-fed spec. diet	Fasting 1st 4 days		Fasting 7th–11th days		Fasting 14th–last days	
					Rats	*Rev.*	*Rats*	*Rev.*	*Rats*	*Rev.*
				Experiment 1						
Carbohydrate	8	618	Diet not fed	227	8	169		Not measured		
Fat	8	543	Diet not fed	306	8	318		Not measured		
Protein	3	500	Diet not fed	192	3	135		Not measured		
				Experiment 2						
Carbohydrate	7	540	608	308	6	366	5	238	5	639
Fat	6	626	545	366	6	319	5	89	4	154
Protein	6	600	599	253	5	372				
				Experiment 3						
Carbohydrate	5	632	Diet not fed	382	4	312	3	355	3	671
Fat	4	566	Diet not fed	222	4	110	4	34	3	532
Protein	5	749	Diet not fed	266	1	63	1	530		
				Experiment 4						
Carbohydrate	7		Diet not fed	359				Not measured		
Fat	5		Diet not fed	225				Not measured		
Protein	6		Diet not fed	251				Not measured		
				Composite:						
Carbohydrate	20	598 ± 98		308 ± 33	18	257 ± 45	8	253 ± 58	8	643 ± 120
Fat	18	569 ± 82		285 ± 32	18	272 ± 39	9	65 ± 25	7	192 ± 30
Protein	13	642 ± 130		250 ± 23	9	284 ± 90				
				All force-fed mixed diet						
	16		602 ± 133							

Spontaneous activity, forced activity and survival during fasting

In table 3 it will be seen that there is a highly significant difference between mean periods of survival for the rats on the different diets. This was in spite of an equicaloric intake during the feeding period. The rats on the high fat diet survived the longest, 18.3 days. The carbohydrate-fed

TABLE 3

Exercise causing exhaustion of adult male rats, nitrogen excreted and survival while fasting with periodic exercise

	CARBOHYDRATE		FAT		PROTEIN	
	Rats	Category of interest	Rats	Category of interest	Rats	Category of interest
Exercise,[1] 2–4 days fasting (Revolutions)	16	4040 ± 411	12	6730 ± 915	7	3600 ± 262
Exercise,[1] 9–11 days fasting (Revolutions)	14	8660 ± 1017	13	8800 ± 1020	4	7220 ± 500
Exercise,[1] 16–18 days fasting (Revolutions)	3	8650	7	7960 ± 738	0	
Total forced exercise fasting (Revolutions)	14	13800 ± 1630	12	19700 ± 1780	9	6465 ± 1650
Nitrogen excreted per unit work (mg/1000 rev.)	19	.0465 ± .0058	22	.0342 ± .0044	11	.0521 ± .0095
Fasting survival (days)	13	15.5 ± 0.46	12	18.3 ± 0.44	9	10.2 ± 1.68

[1] Rate of exercise was 7 r.p.m. or 20 ft. per min.

animals were next, with an average survival of 15.5 days. This was significantly shorter than that of the fat-fed rats (P less than 0.01) and definitely longer than that of the animals fed protein (P less than 0.01).

When the rats were forced to exercise to exhaustion at some time during the first 5 days, those receiving the high

fat diet ran significantly longer than the carbohydrate-fed group, the mean difference being 2690 revolutions (6730 vs. 4040). This was also significantly longer than the average for the protein-fed rats (6730 vs. 3600). In the second and third exercise periods no significant difference existed. The number of animals surviving for repeated exhaustion trials dropped markedly in the case of the protein-fed group by the time of the second test, and in the case of the carbohydrate-fed group by the third test. There was therefore some selective action. When the mean total forced activity was determined (sum of all exercise tests through which the animal survived), there were significant differences among all three groups, the fat-fed rats being best, those previously on a carbohydrate diet next, and the high protein-fed animals poorest.

One of the interesting observations was that the time required for exhaustion of the rats on any of the diets increased when the second test was run. The change was not so great among the fat-fed animals as in the carbohydrate-fed group. It was impossible to make any quantitative comparisons between the protein-fed rats and the others on the basis of the second exercise period, since so few animals previously on the high protein diet survived long enough. This increased time for exhaustion did not seem to be the result of a simple learning process, since animals exercised immediately at the end of the feeding period (experiment 3) did not show an increase on their next fasting test.

Not only was there an increase in the amount of work required for exhaustion as the fast lengthened, but terminally there was also an increase in the voluntary activity. This was not an immediate effect of inanition, however, since the voluntary activity rates during the first 4 days of fasting did not differ significantly from those for animals on the diets. In fact, the voluntary activity of the rats previously fed a high fat diet gradually decreased, and reached a low point between the 7th and 11th days. As death approached, however, these rats also showed a spurt in voluntary activity.

While the rats previously fed the high fat diet were able to do significantly more work before exhaustion than either of the other groups, their voluntary activity was less. As a consequence, the total activity of the fat-fed rats during fasting, although averaging slightly more, did not differ significantly from that of the carbohydrate-fed group. Both differed significantly from the protein-fed rats because of the high incidence of early deaths among the latter.

Changes in blood sugar during fasting and exercise

The changes in blood sugar levels during fasting are shown in figure 1. Values after forced activity are not included. During the first 6 hours after feeding the glucose level of the

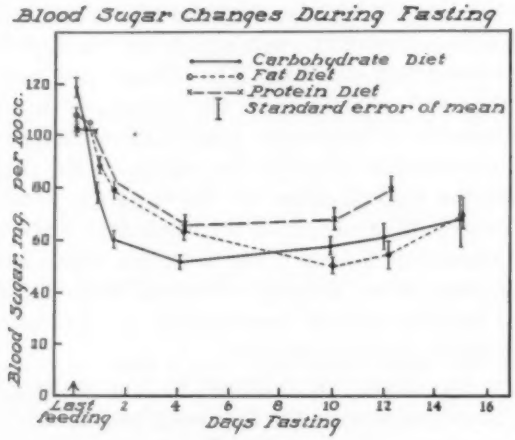

Figure 1

carbohydrate-fed rats was significantly higher than that of the other two groups. It also fell more rapidly in this group, however, so that 8–12 hours after feeding there was no significant difference. By 16 hours after feeding the order had been reversed, the carbohydrate-fed rats having significantly lower blood sugar levels. This difference was still

12 mg per 100 ml at the end of 4 days of fasting, and was highly significant statistically.

By the 10th day, however, the difference had disappeared. In fact, the carbohydrate and protein groups had now apparently higher blood sugar levels than the fat-fed animals. This was due to the fact that more of the animals in the first two groups were approaching death. During the 48 hours preceding death, the blood sugar level rose in all animals. This was also the period of increased voluntary activity; probably the two phenomena are causally related, perhaps via the adrenals.

TABLE 4

Blood sugar changes during forced exercise

	CARBOHYDRATE	FAT	PROTEIN
		mg/100 ml blood	
First exercise period:			
Blood sugar before	55.5 ± 2.8	62.4 ± 2.8	66.2 ± 5.4
Blood sugar at exhaustion	58.9 ± 5.4	88.9 ± 6.3	73.0 ± 8.9
Difference	+ 3.4 ± 6.0	+ 26.5 ± 6.2	+ 6.8 ± 7.1
Second exercise period:			
Blood sugar before	59.9 ± 3.6	57.7 ± 5.7	69.5 ± 6.6
Blood sugar at exhaustion	58.6 ± 4.1	76.6 ± 8.6	72.2 ± 14.2
Difference	— 1.3 ± 5.5	+ 18.9 ± 9.2	+ 2.7 ± 17.2

There were marked differences in the effects of exercise on the blood sugar levels (table 4). When samples were taken at the beginning of forced exercise and at exhaustion, the rats originally fed carbohydrate showed small and irregular differences among themselves. The mean change was a slight increase of 3.4 mg per 100 ml. In the protein-fed group the change was similar. On the other hand, the rats previously fed a high fat diet, with rare exceptions, showed a considerable increase in the blood sugar level during exercise. The average increase was 26.5 mg per 100 ml. Apparently there was a marked difference between the two groups of animals with respect to the balance between glucose production and utilization.

Nitrogen excretion during feeding, fasting and exercise

Nitrogen excretion was measured during the last 5 days on the special diets. As shown in table 5, the nitrogen excretion bore a direct relation to the nitrogen intake and in all groups showed a slightly positive balance consistent with the increasing size of the animals.

During fasting, however, there were significant differences in excretion due to the diets previously fed. In the first 4 days the rats previously fed protein excreted significantly more nitrogen and the fat-fed rats significantly less than

TABLE 5

Nitrogen excretion on special diets and during subsequent fasting with voluntary activity

DIET	ON DIET	1ST FASTING PERIOD (0–4th days)	2ND FASTING PERIOD (6th–11th days)	3RD FASTING PERIOD (13 day–death)
		gm. N per rat per day		
High carbohydrate	0.3375 ± .0053	0.1770 ± .0068	0.1318 ± .0111	0.2286 ± .0131
High fat	0.3165 ± .0080	0.1104 ± .0057	0.1004 ± .0094	0.1407 ± .0272
High protein	1.369 ± .038	0.2547 ± .0314	0.1427 ± .0392	

those of the carbohydrate-fed group. The nitrogen excretion of the carbohydrate-fed group continued to drop, while that of the fat-fed group tended to level off; the difference between them therefore decreased. Terminally there was some increase in the nitrogen output of all animals, the so-called "premortal rise."

Not only was there a significant difference in the nitrogen excretion during fasting associated with voluntary activity, but the fat-fed rats also excreted less nitrogen during the exercise periods. As may be seen in table 3, the nitrogen output per unit of work was definitely lower for these animals. They must therefore have been burning more non-protein com-

pounds. These results agree with the hypothesis that for some time after feeding has ceased the tissues of the carbohydrate-fed rats tend to use glucose more readily for energy, and fat less readily, than do those of the fat-fed rats. Since carbohydrate storage is relatively small, there is a greater tendency to mobilize protein to supply similar molecular fragments.

DISCUSSION

Two factors appear to be involved in the longer survival of the fat-fed rats in the present experiments: (1) a decreased destruction of structural tissue and (2) a reduced amount of voluntary activity.

The decreased destruction of structural tissue during any given period of fasting was due in part to the reduced voluntary activity. This is probably not the entire explanation, however. The early course of the blood sugar level would indicate that in all probability during the first part of the fast a different metabolic mixture was burned by the animals previously on the different diets. In the case of the carbohydrate group, this involved greater carbohydrate and protein and less fat breakdown. The greater utilization of fat and the consequent sparing of body protein during forced exercise probably accounts for the greater work which the fat-fed rats were able to do before exhaustion. This beneficial effect of a fat diet has also been observed by Deuel et al. ('47) in connection with a high fat diet fed ad libitum.

Differences in glucose utilization, measured by glucose tolerance tests or glycosuria after ingestion of carbohydrate, have been observed by many workers since Hofmeister wrote of "hunger diabetes" as far back as 1890. The literature has been reviewed by Chambers ('38) and by Peters ('45). The effects of a high fat diet and of fasting were formerly thought to be the same, however. The present work demonstrates that the effects of a high fat or high carbohydrate diet persist over a large part of the subsequent fasting survival

period, and are therefore superimposed upon the metabolic changes due to fasting alone.

The recent report of Lundbaek and Stevenson ('48) indicates that the voluntary muscle of a carbohydrate-fed rat utilizes glucose *in vitro* at twice the rate of that of the fat-fed animal when both are exposed to the same environment. Apparently this excess utilization is for energy, since glycogen storage was the same in both groups. Gilmore and Samuels ('48) of this laboratory have confirmed the difference in glucose utilization by diaphragmatic muscle and have shown that a similar difference prevails in the rate of glycogen disappearance when diaphragms from carbohydrate- or fat-fed rats are incubated aerobically in a glucose-free medium. It would appear, therefore, that the local cellular metabolic system is affected.

The increase in the blood sugar level of the previously fat-fed rats during exercise is probably explained by the stimulation of glucose formation from glycogen due to epinephrine release, and by the stimulating effect of exercise on the release of the 11-oxygenated steroids from the adrenal. These increase the breakdown of protein to carbohydrate and at the same time decrease glucose oxidation. Probably both types of hormones were released in both groups of rats, but in the fat-fed group the oxidation of carbohydrate was already decreased at the expense of fat; the glucose mobilized by hormones therefore accumulated. In the carbohydrate-fed group the oxidation of glucose was sufficient to leave the animal in glucose balance.

The marked effect on voluntary activity which followed the change from a mixed diet to one having one major source of energy raises the question of the influence of mixed diets on exercise and survival during fasting. Perhaps the omnivore is in an optimum state when the diet is a mixed one rather than when any single foodstuff predominates. If, however, one foodstuff is to constitute the major source of energy, it would appear that chances for survival under fasting and stress are best when a high fat diet has been eaten.

CONCLUSIONS

With the rat as the experimental animal the administration of a high fat diet (fat furnishing 80% of calories) led to longer survival during subsequent fasting and the ability to do greater amounts of work before exhaustion than a high carbohydrate or high protein diet fed in equicaloric amounts. The protein-fed rats survived for the shortest period.

The difference between the fat and carbohydrate groups could be accounted for on the basis of the difference in the metabolic mixture used and the lower voluntary activity of the fat-fed animals. The early deaths of rats previously on a high protein diet were not explained by these experiments.

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MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1949 Award of $1000, established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the spring of 1949.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year, January 1 to December 31, the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1948 must be in the hands of the Chairman of the Nominating Committee by January 15, 1949. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

HAROLD H. WILLIAMS
Cornell University, Ithaca, N. Y.

CHAIRMAN, NOMINATING COMMITTEE

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1949 Award of $1000, and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1949. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 15, 1949. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

JAMES M. ORTEN
College of Medicine,
Wayne University,
Detroit, Michigan

CHAIRMAN, NOMINATING COMMITTEE

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000, established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1949 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 15, 1949.

D. W. Woolley
Rockefeller Institute for Medical Research, New York, N. Y.

Chairman, Nominating Committee